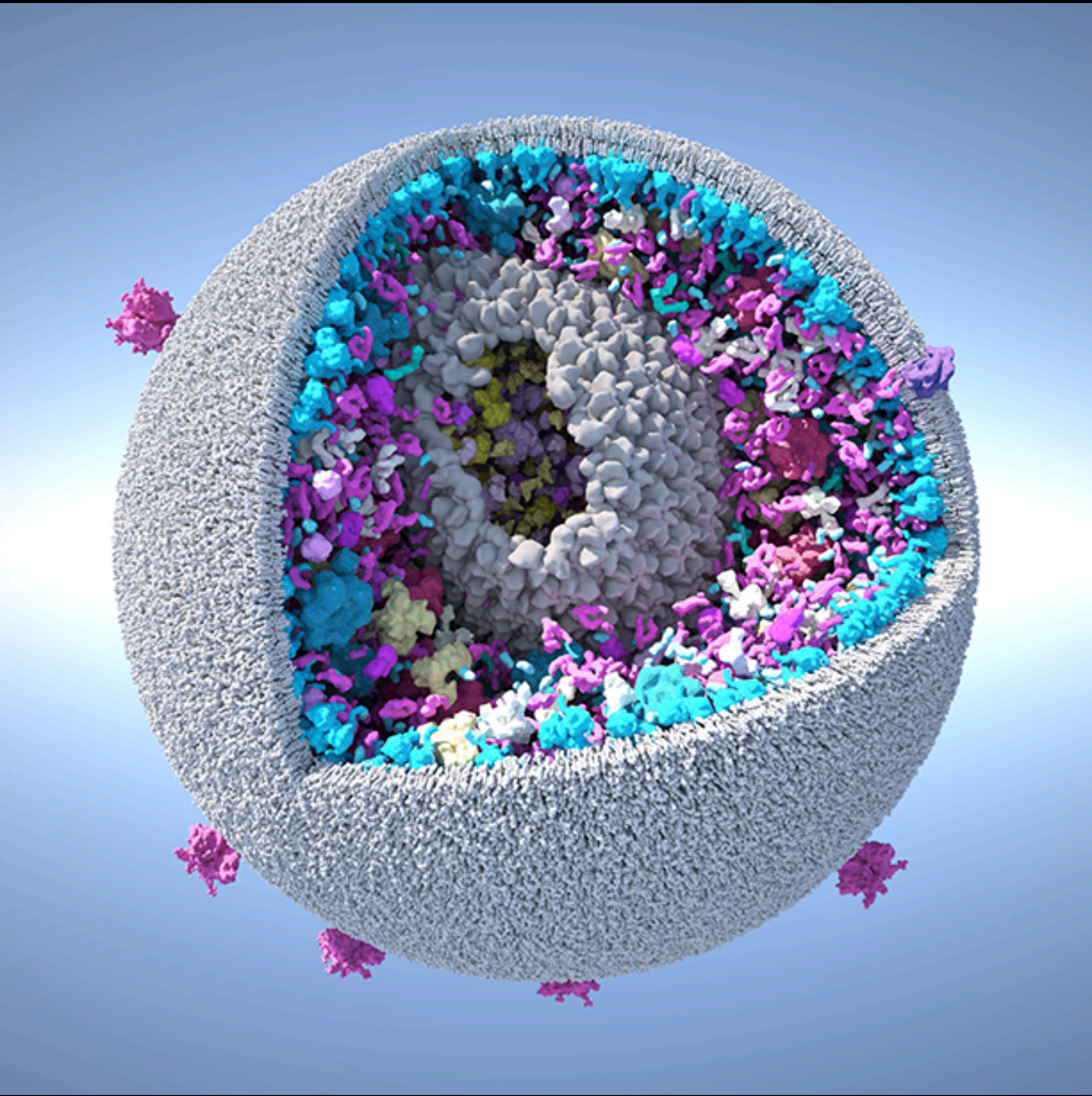
Structural Biology Related to HIV/AIDS - 2015



Thursday, June 18 – Friday, June 19, 2015
Ruth L. Kirschstein Auditorium

Natcher Conference Center
Bethesda, Maryland

NIH National Institute of General Medical Sciences

NIH National Institute of Allergy and Infectious Diseases

About the Cover:

3D rendering of a model of the mature HIV virion at the molecular level by Ludovic Autin in the Olson Laboratory, The Scripps Research Institute. The model was generated using cellPACK[1] using a detailed recipe of all ingredients[2]. The lipids membrane bilayer was generated using LipidWrapper[3]. The model was rendered in the Maxwell Render software[4].

1. Johnson GT, Autin L, Al-Alusi M, Goodsell DS, Sanner MF, Olson AJ (2015), cellPACK: a virtual mesoscope to model and visualize structural systems biology. Nature Methods 12, 85–91 (2015), PubMed PMID: 25437435
2. Johnson GT, Goodsell DS, Autin L, Forli S, Sanner MF, Olson AJ (2014), 3D molecular models of whole HIV-1 virions generated with cellPACK. *Faraday Discuss*, 169, 23-44. PubMed PMID: 25253262
3. Durrant JD, Amaro RE (2014) LipidWrapper: An Algorithm for Generating Large-Scale Membrane Models of Arbitrary Geometry. PLoS Comput Biol 10(7): e1003720. doi:10.1371/journal.pcbi.1003720
4. www.maxwellrender.com

Table of Contents

BACK

Structural Biology Related to HIV/AIDS – 2015

DAY 1

8:00 – 8:10 **Michael Sakalian** (National Institute of General Medical Sciences)
Opening Remarks

Session I: Reports from Specialized Centers (P50); Session Chair: Celia Schiffer

8:10 – 9:10 The Center for the Structural Biology of Cellular Host Elements in Egress, Trafficking, and Assembly of HIV (CHEETAH)

Wesley I. Sundquist (University of Utah School of Medicine)
Release and Uptake of Designed Enveloped Nanoparticles

Barbie Ganser-Pornillos (University of Virginia)
CryoEM Structure of a T=4 Icosahedral HIV-1 Capsid-Like Shell

Walther Mothes (Yale University School of Medicine)
Retrovirus Dissemination Depends on CD169/Siglec-1 Mediated Trans-Infection of Permissive Lymphocytes

9:10 – 10:10 The Pittsburgh Center for HIV Protein Interactions (PCHPI)

Angela M. Gronenborn (University of Pittsburgh School of Medicine)
Progress update from the Pittsburgh Center for HIV Protein Interactions

Peijun Zhang (University of Pittsburgh School of Medicine)
Structure of mature HIV-1 capsid and its interaction with cyclophilin A

Peter Cherepanov (The Francis Crick Institute)
Structural basis for retroviral integration into nucleosomes

10:10 – 10:40 BREAK

Session II: Reports from Specialized Centers (P50) continued; Session Chair: Alan Frankel

10:40 – 11:40 Center The HIV Interaction and Viral Evolution Center (HIVE)

Arthur Olson (The Scripps Research Institute)
HIVE Center: HIV Interactions and Viral Evolution of Drug Resistance

Karin Musier-Forsyth (Ohio State University)
Unexpected Connections of tRNA Synthetases to HIV-1 Replication: Implications for tRNA Primer Recruitment and Initiation of Reverse Transcription

Alan Engleman (Dana-Farber Cancer Institute) and **Dmitry Lyumkis** (Salk Institute for Biological Studies)
Structural and Functional Characterization of the Mouse Mammary Tumor Virus Intasome

Eddy Arnold (Rutgers University)
HIV-1 Reverse Transcriptase Complexed with a Novel 38-mer Hairpin Template-Primer DNA Aptamer: Platform for Structural and Mechanistic Investigations

Session III: Special Presentation on Communicating Science; Session Chair: Michael Sakalian, NIGMS

11:40 – 12:10 **David Goodsell** (HIVE; The Scripps Research Institute) **& Janet Iwasa** (CHEETAH; University of Utah)
Visualization and Animation of the HIV Life Cycle

12:10 – 1:00 LUNCH

1:00 – 3:00 POSTER SESSION

Session III: Reports from Specialized Centers (P50) continued; Session Chair: Wes Sundquist

3:00 – 4:00 The Center for HIV RNA Studies (CRNA)

Alice Telesnitsky (University of Michigan)
Recruitment of HIV Genomic RNA

Wah Chiu (Baylor College of Medicine)
CryoEM of HIV RNA

Victoria D'Souza (Harvard University)
A Dual Structural Mimicry Enables Expression of the HIV-1 Genome

4:00 – 5:00 The Center for HIV Accessory and Regulatory Complexes (HARC)

Nevan Krogan (University of California San Francisco)
The HARC Center: Progress and Overview

Qing-Tao Shen (University of California Berkeley)
HIV-1 Nef Hijacks Clathrin Coats by Stabilizing AP-1 Polygons

John D. Gross (University of California San Francisco)
Resolution and Inhibition of Vif-APOBEC3 Interactions

5:00 ADJOURN FOR DAY

DAY TWO

Session IV: Assembly, Session Chair: Alice Telesnitsky

8:00 – 8:30 **Wei-Shau Hu** (NCI HIV Dynamics and Replication Program)
HIV-1 RNA genome: the journey and the destination

8:30 – 9:00 **Don Lamb** (Ludwig Maximilians University of Munich)
The Onset of Viral Assembly and the Interaction of Endogenous ESCRT Proteins with HIV Assembly Sites Investigated Using Advanced Fluorescence Microscopy

9:00 – 9:30 **Boon Chong Goh** (University of Illinois)
Computational Modeling of the Immature Retroviral Lattice

9:30 – 10:00 **Stefan G. Sarafianos** (University of Missouri Columbia)
Crystal Structures of Native and Mutant HIV-1 Capsid Proteins Reveal Molecular Details of Interactions with Ligands and Structural Basis of Capsid Stability

10:00 – 10:30 BREAK

Session V: APOBEC, Session Chair: Arthur Olson

10:30 – 11:15 P01: Critical Interactions of APOBEC3s: Molecular Approaches to Novel HIV Therapies

Celia Schiffer (UMass Medical School)
Structural Studies of APOBECs

Hiroshi Matsuo (University of Minnesota)
Structure of the Vif-Binding Domain of APOBEC3G

Daniel Harki (University of Minnesota)
Chemical Probes of APOBEC3G Deamination

Session VI: Resistance to PR Inhibitors, Session Chair: Irwin Chaiken

11:15 – 12:00 P01: The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease

Ronald Swanstrom (UNC Chapel Hill School of Medicine)
A Direct Interaction With RNA Dramatically Enhances the Catalytic Activity of the HIV-1 Protease In Vitro

Woody Sherman (Schrodinger, Inc.)
Free Energy Perturbation Predicts the Energetics of Resistance

Celia Schiffer (University of Massachusetts Medical School)
Mutations Outside the Active Site and Co-Evolution of Substrates Confer Drug Resistance Through Alterations in the Dynamic Network and Structural Ensemble of HIV-1 Protease

12:00 – 1:00 LUNCH
(SAB Lunch in 2As.10; Please bring your lunch to the second floor.)

1:00 – 3:00 POSTER SESSION
(1:00 – 3:00 NIGMS Centers Scientific Review Board; Room 2As.10; second floor)

Session VIII: Structure of Early Events, Session Chair: Elizabeth Church

3:00 – 3:30 **Tracy Handel** (University of California, San Diego)
The Structure of a CXCR4:Chemokine Complex with Insights into Chemokine Receptor Recognition by gp120

3:30 – 4:00 **Hideki Aihara** (University of Minnesota)
Structure of the Rous Sarcoma Virus Intasome

Session VII: Envelope, Session Chair: Angela Gronenborn

4:00 – 4:45 P01: Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry

Irwin Chaiken (Drexel University College of Medicine)
Advancements in Env gp120 Inhibitor Designs and Structural Mechanisms

Navid Madani (Dana Farber Cancer Institute)
Sensitization of HIV-1 to Vaccine-Elicited Antibodies by Small Molecule CD4 Mimetics

Walther Mothes (Yale University)
Conformational Dynamics of Single HIV-1 Env Trimers on Native Virions

4:45 ADJOURN MEETING

Mark Your Calendars!

Structural Biology Related to HIV/AIDS – 2016

Thursday June 23 – Friday June 24, 2016
Natcher Conference Center, Bethesda, Maryland

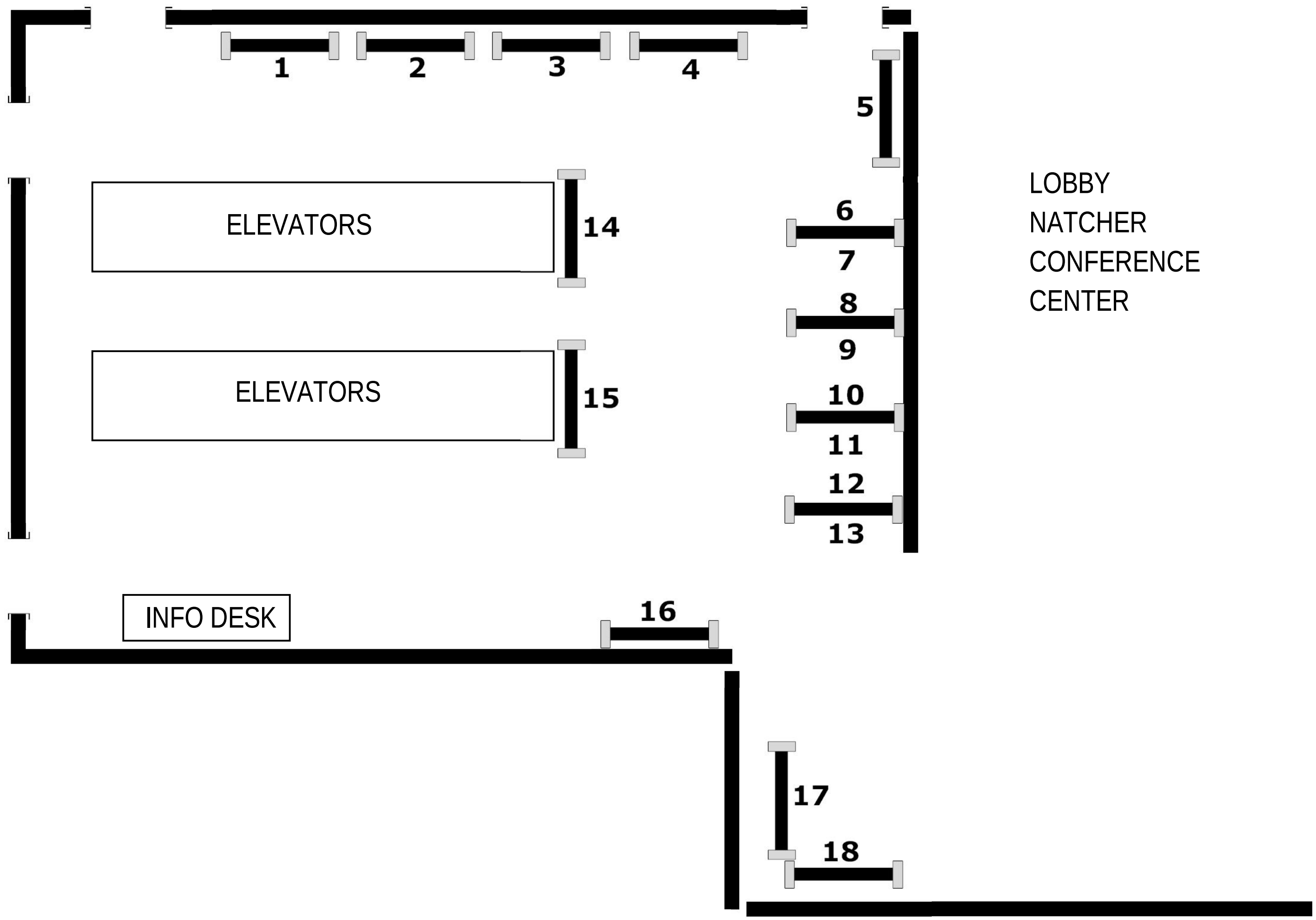
BACK

About the Poster Sessions

Thursday	**1:00 – 3:00**	**T-numbered posters**
Friday	**1:00 – 3:00**	**F-numbered posters**

Location maps for posters follow this page. Please remove your poster from the board at the end of the assigned day. There are too many for posters to be left up for the whole meeting.

BACK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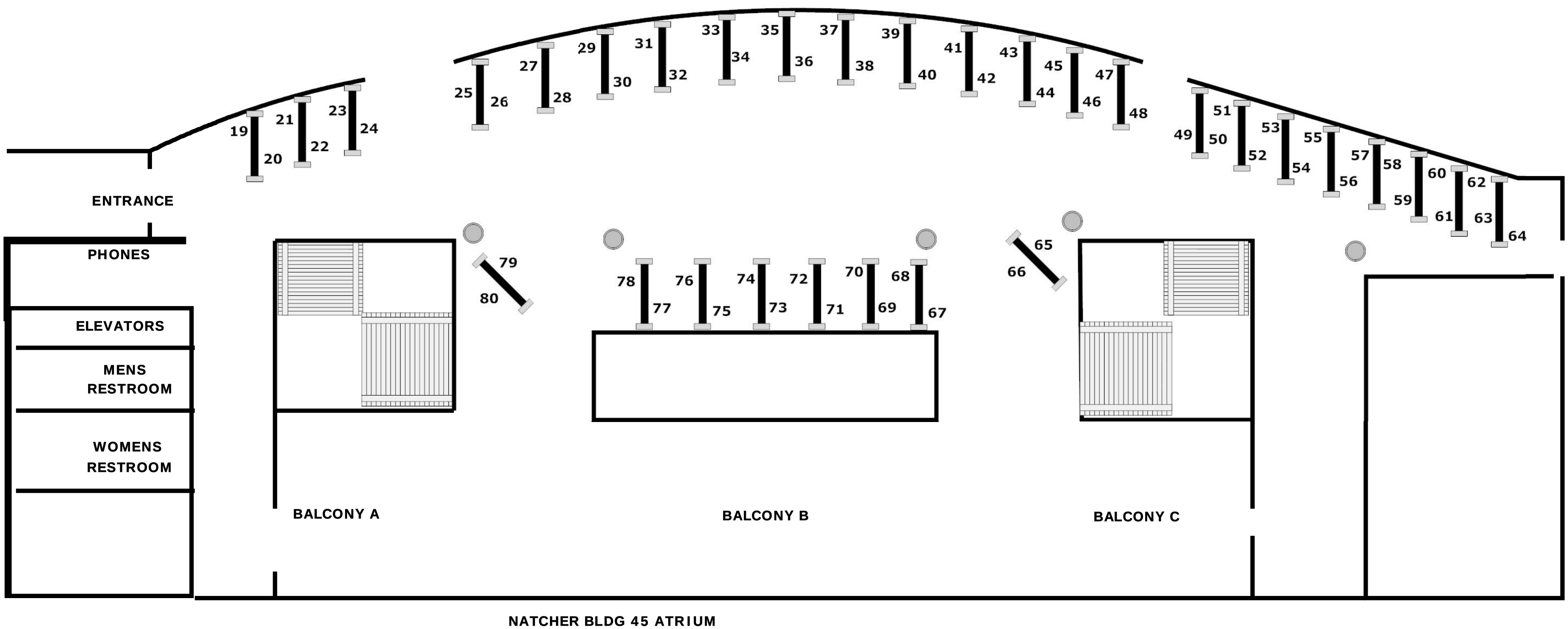
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49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64
65 66
67 68 69 70 71 72 73 74 75 76 77 78
79 80
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BACK

Thursday Posters

Transmission and Entry

T1. Retrovirus Dissemination Depends on CD169/Siglec-1 Mediated *Trans*-Infection of Permissive Lymphocytes

Xaver Sewald[1], Mark S. Ladinsky[2], Pradeep D. Uchil[1], Jagadish Beloor[3], Ruoxi Pi[1], Thomas T. Murooka[4], Christin Herrmann[1], Nasim Motamedi[1], Michael A. Brehm[5], Dale L. Greiner[5], Leonard D. Shultz[6], Thorsten R. Mempel[4], Pamela J. Bjorkman[2], Priti Kumar[3], Walther Mothes[1]
[1]Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT 06510; [2]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125; [3]Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06510; [4]Center for Immunology and Inflammatory Diseases, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114; [5]Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01655; [6]The Jackson Laboratory, Bar Harbor, ME 04609

T2. Characterization of Novel Mutations in the HIV-1 Env Glycoprotein that Globally Rescue Defects in Virus Replication

Rachel Van Duyne, Lillian Kuo, Ken Fujii, and Eric O. Freed
Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD

T3. Incorporating Cheminformatic Predictive Tools into Structure-Based Discovery of CCR5 Antagonists

Terry-Elinor Reid, Ronald Smith, Abdullah Zubais, and Xiang Simon Wang
Molecular Modeling and Drug Discovery Core for District of Columbia Developmental Center for AIDS Research (DC D-CFAR); Laboratory of Cheminfomatics and Drug Design, Department of Pharmaceutical Sciences, College of Pharmacy, Howard University, Washington, District of Columbia 2005

BACK

Envelope: gp120 and Inhibitors

T4. Structural Targeting of Potentially Protective HIV-1 gp120 Epitopes in the C1-C2 Region

Neelakshi Gohain[1,2], William D. Tolbert[1,2], Chiara Orlandi[1,3], Maxime Veillette[1,4], Jean-Philippe Chapleau[1,4], Maria Visciano[1,3], Andres Finzi[1,4], George K. Lewis[1,3], Marzena Pazgier[1,2]
[1]Institute of Human Virology and Departments of [2]Biochemistry and Molecular Biology, [3]Department of Microbiology and Immunology of University of Maryland School of Medicine, Baltimore, MD 21201, USA; [4]Centre de Recherche du Centre hospitalier de l'Université de Montréal and Department of Microbiology, Infectiology and Immunology, Université de Montréal, Quebec, Canada

T5. Microscopic Detection and Localization of HIV gp120 Epitope Exposures on Cell-Bound Virions

Meron Mengistu, George K. Lewis, and Anthony L. DeVico
Institute of Human Virology of the University of Maryland School of Medicine, Baltimore, Maryland, 21201

T6. Understanding Glycan Type Specificity in Highly Glycosylated Proteins

Artem Krantsevich and David F. Green
Stony Brook University

T7. Optimization of Small-Molecule CD4-Mimetic HIV-1 Entry Inhibitors

Francesca Moraca[1,2,3], Bruno Melillo[3], Cameron F. Abrams[1,2], Wayne Hendrickson[4], Shuaiyi Liang[4], Navid Madani[5], Joseph Sodroski[5,6], and Amos B. Smith, III[3]
[1]Department of Chemical and Biological Engineering, Drexel University, Philadelphia, Pennsylvania; [2]Department of Biochemistry and Molecular Biology, Drexel University, Philadelphia, Pennsylvania; [3]Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania; [4]Department of Biochemistry and Molecular Biophysics and Department of Physiology and Cellular Biophysics, Columbia University, New York, New York; [5]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts; [6]Department of Microbiology and Immunology, Harvard Medical School and Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts

T8. Investigating Lectin Binding to Envelope Glycoprotein gp120

Chelsea Kennedy and David F. Green
Stony Brook University

T9. Macrocyclic HIV-1 Envelope Glycoprotein Antagonists

Adel A. Rashad[1], Ramalingam Venkat Kalyana Sundaram[1,2], Aakansha Nangarlia[1,2], Rachna Aneja[1], Caitlin Duffy[1] and Irwin Chaiken[1]
[1]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [2]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104

T10. Mapping the Conformational Space of Glycoconjugate-Linked Carbohydrates

Xindi Li[1] and David F. Green[1, 2, 3, 4]
[1]Department of Applied Mathematics & Statistics, [2]Department of Chemistry, [3]Laufer Center for Physical and Quantitative Biology, [4]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794-3600, United States

T11. Interaction Analysis of HIV BG5O5.SOSIP.664 Trimer with Peptide Triazole UM15

Kriti Acharya[1], Adel Ahmed[1], Francesca Moraca[2], Cameron Abrams[2], and Irwin Chaiken[1]
[1]Dept. Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19102; [2]Dept. Chemical and Biological Engineering, Drexel University, Philadelphia, Pennsylvania 19104

T12. Understanding Dynamic Structural Variations in HIV-1 Envelope Glycoprotein gp120

Tuoling Qiu[1] and David F. Green[1, 2]
[1]Chemistry Department, Stony Brook University, Stony Brook, NY 11794; [2]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY 11794

T13. Targeting Cell Surface gp120 with Peptide Triazoles Can Suppress Infectious HIV-1 Formation and Inactivate Virus Producer Cells

Arangassery Rosemary Bastian[1,2*≠], Charles G. Ang[1,2*], Kantharaju Kamanna[1¶], Farida Shaheen[3], Yu-Hung Huang[1], Karyn McFadden[1†], Caitlin Duffy[1], Lauren D. Bailey[1], Ramalingam Venkat Kalyana Sundaram[1,2], and Irwin Chaiken[1]
[1]Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [2]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104; [3]Viral and Molecular Core, Penn Center for AIDS Research, Philadelphia, PA 19104

BACK

Envelope: gp41 and Inhibitors

T14. CD4-Env Interaction Stoichiometry Controls the Spatial and Temporal Exposure of the gp41 Trimer

Mukta Khasnis, Konstantine Halkidis, Anshul Bhardwaj, and Michael Root
Thomas Jefferson University

T15. Investigation into the HIV-1 Envelope Lipid-Protein Machine Responsible for the Virolytic Effects of Peptide Triazole Thiols

R Venkat Kalyana Sundaram[1,2], Huiyuan Li[3], Lauren Bailey[1], James Huynh[4], Rachna Arora[1], Karl Weiss[5], Rosemary Bastian[1,2], Cameron Abrams[5], Steven Wrenn[5] and Irwin Chaiken[1]
[1]Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102; [2]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA 19104; [3]Shared Research Facilities, West Virginia University, Morgantown, WV 26506; [4]Department of Biology and [5]Department of Chemical and Biological Engineering, Drexel University, Philadelphia, PA 19104

T16. Free-Energy Protocol to Aid the Design Small-Molecule HIV Fusion Inhibitors

T. Dwight McGee Jr.[1], William J. Allen[1], and Robert C. Rizzo[1,2,3,]
[1]Department of Applied Mathematics & Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York

T17. Highly Potent, Broad Anti-HIV-1 D-Peptide Entry Inhibitor: Development and Preclinical Characterization

J. Nicholas Francis[1], Joseph S. Redman[2], Damon Papac[1], Alan L. Mueller[1], Brett D. Welch[1], Debra M. Eckert[2] and Michael S. Kay[2]
[1]D-Peptide Research Division, Navigen, Inc., Salt Lake City, UT; [2]Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT

T18. Design and Refinement of HIV Inhibitors Using DeNovo DOCK

Brian C. Fochtman[1], William J. Allen[2], and Robert C. Rizzo[2,3,4]
[1]Department of Biochemistry and Cellular Biology, [2]Department of Applied Mathematics & Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

T19. Complex Mechanism of Synergy between HIV-1 Entry Inhibitors

Koree W. Ahn, and Michael J. Root
Thomas Jefferson University, United States of America

T20. Origins of Resistance of T20 with HIVgp41 Using Molecular Dynamics Simulations

Lingling Jiang[1] and Robert C. Rizzo[1,2,3]
[1]Department of Applied Mathematics & Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

Uncoating and TRIM5α

BACK

T21. Ebselen, a Small Molecule Inhibitor of HIV-1 Capsid Dimerization

Suzie Thenin-Houssier[1], Ian M. De Vera[2], Laura Pedro-Rosa[3], Angela Brady[1], Briana Konnick[1], Audrey Richard[1], Timothy Tellinghuisen[1], Hyeryun Choe[1], Timothy Spicer[3], Douglas J. Kojetin[2], and Susana T Valente[1]
[1]Department of Immunology and Microbial Sciences, The Scripps Research Institute, Florida, USA; [2]Department of Molecular Therapeutics, The Scripps Research Institute, Florida, USA; [3]Molecular Screening Center, The Scripps Research Institute, Florida, USA

T22. Live Cell Imaging of HIV Uncoating

João I Mamede and Thomas J Hope
Northwestern University, Department of Cell and Molecular Biology, Chicago, IL

T23. Single-Molecule FRET Reveals Dynamic Conformational Changes in The Rhesus TRIM5α Dimer

Rajan Lamichhane[1], Santanu Mukherjee[2], Nikolai Smolin[3], Seth Robia[3], David Millar[1], and Edward M Campbell[2]
[1]Scripps Research Institute, La Jolla, CA, USA; [2]Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University Chicago, Chicago, IL, USA; [3]Department of Physiology, Stritch School of Medicine, Loyola University Chicago, Chicago, IL, USA

T24. RING Dimerization Couples Higher-Order Assembly of TRIM5α to Synthesis of K63-Linked Polyubiquitin

Zinaida Yudina[1], Rory Johnson[1], Amanda Roa[2], Nikolaos Biris[1], Vladislav Tsiperson[2], Alexander Taylor[1], P. John Hart[1], Borries Demeler[1], Felipe Diaz-Griffero[2], and Dmitri N. Ivanov[1]
[1]Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78229; [2]Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461

RT Structure and Inhibition

BACK

T25. Asymmetric Conformational Maturation of HIV-1 Reverse Transcriptase

Xunhai Zheng, Lalith Perera, Geoffrey A Mueller, Eugene F DeRose, and Robert E London
Genome Integrity and Structural Biology Laboratory, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, United States

T26. A Role-Reversal for the p66 and p51 Subunits of HIV-1 Reverse Transcriptase

Xunhai Zheng, Geoffrey A. Mueller, Eugene F. Derose and Robert E. London
Genome Integrity and Structural Biology Laboratory, NIEHS, NIH, Research Triangle Park, NC 27709

T27. Structure of HIV-1 Reverse Transcriptase Bound to a Novel 38-mer Hairpin Template-Primer DNA Aptamer

Matthew T. Miller[1], Steve Tuske[1], Kalyan Das[1], Irani Ferreira-Bravo[2], Jeffrey DeStefano[2], and Eddy Arnold[1,*]
[1]*Center for Advanced Biotechnology and Medicine, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854, USA;* [2]*Department of Cell Biology and Molecular Genetics, University of Maryland College Park, College Park, Maryland 20742, USA.*

T28. Cryo-Electron Microscopy of Retroviral Initiation and Integration Complexes

Eddy Arnold[1], Allison Ballandras-Collas[2], Monica Brown[3], Alan Engelman[2], Dmitry Lyumkis[3], Steve Tuske[1]
[1]*Center for Advanced Biotechnology and Medicine and Department of Chemistry and Chemical Biology, Rutgers University, 679 Hoes Lane West, Piscataway, New Jersey 08854, USA;* [2]*Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA, USA;* [3]*Laboratory of Genetics, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA*

T29. Subunit-Specific ^{15}N-Isotopic Enrichment of HIV-1 Reverse Transcriptase Facilitates Unambiguous Peptide Identification During Hydrogen-Deuterium Exchange Coupled to Liquid Chromatography-Mass Spectrometry (HDX-MS): Applications to Drug Binding and Mapping Nucleic Acid Contacts

Steve Tuske[1], Devrishi Goswami[2], Jerry Joe Harrison[1], Will Cantara[3], Erik Olson[3], Joe Bauman[1], Disha Patel[1], Bruce Pascal[1], Karin Musier-Forsyth[3], Patrick Griffin[2], and Eddy Arnold[1]
[1]*Center for Advanced Biotechnology and Medicine, and Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ;* [2]*The Scripps Research Institute, Department of Molecular Therapeutics, Jupiter, FL;* [3]*Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retrovirus Research, The Ohio State University, Columbus, OH*

T30. Effect of Nucleic Acid Sequence on the DNA Polymerization and NNRTI Inhibition Mechanisms of HIV-1 Reverse Transcriptase

Obiaara Ihenacho[1,2], Andrew D. Huber[1,3], Eleftherios Michailidis[1,2], Briana Bester[1,2], Kaylan Das[4,5], Michael A. Parniak[6], Kamlendra Singh[1,2], Eddy Arnold[4,5], and Stefan G. Sarafianos[1,2,7]
[1]*Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA;* [2]*Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, MO 65211, USA;* [3]*Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, USA;* [4]*Center for Advanced Biotechnology and Medicine,* [5]*Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854, USA;* [6]*Department of Microbiology and Molecular Genetics, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA;* [7]*Department of Biochemistry, University of Missouri, Columbia, MO 65211, USA*

T31. Rilpivirine Resistance in HIV-1 Subtype C from Low and Middle Income Countries

Ujjwal Neogi[1], Amanda Häggblom[2], Kamal Singh[3], Shwetha D Rao[1], Amogne Wondwossen[4], Kalyan Das[5], Eddy Arnold[5], Stefan G. Sarafianos[3], and Anders Sönnerborg[1,2]

[1]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institute, Stockholm 141 86, Sweden; [2]Unit of Infectious Diseases, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden; [3]Departments of Molecular Microbiology & Immunology, and Biochemistry, Christopher S. Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, [4]Department of Infection, Immunity and Inflammation, Addis Ababa University, Addis Ababa, Ethiopia, [5]Center for Advanced Biotechnology and Medicine, and Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854

RNase H Structure and Inhibition

BACK

T32. Structural Integrity of the Ribonuclease H Domain in HIV-1 Reverse Transcriptase: pH Dependence Study

Ryan L. Slack[1], Justin Spiriti[2], Michael A. Parniak[3], Daniel M. Zuckerman[2], and Rieko Ishima[1]

Department of Structural Biology[1], Department of Computational and Systems Biology[2], and Department of Microbiology and Molecular Genetics[3], University of Pittsburgh School of Medicine

T33. Structural Features of the HIV-1 Reverse Transcriptase RNase H Domain Reveal a Propensity for Domain Swapping And Unfolding

Xunhai Zheng, Lars C. Pedersen, Scott A. Gabel, Geoffrey A. Mueller, Eugene F. Derose and Robert E. London

Genome Integrity and Structural Biology Laboratory, NIEHS, NIH, Research Triangle Park, NC 27709

T34. Conformational Responses of RNase H of HIV-1 Reverse Transcriptase upon Inhibitor or Ligand Interaction.

Ichhuk Karki[1], Ryan L. Slack[1], Kenji Kanaori[1, 3], Kevin Moy[1], Joon H. Park[1], Nataliya A. Myshakina[2, 4], Michael A. Parniak[2], Zhengqiang Wang[5], and Rieko Ishima[1]

[1]Department of Structural Biology and [2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine; [3]Department of Biomolecular Engineering, Kyoto Institute of Technology, Japan; [4]Science Department, Chatham University; [5]Center for Drug Design, Academic Health Center, University of Minnesota

T35. Re-Purposing HIV-1 Reverse Transcriptase-Associated Ribonuclease H Inhibitors.

Takashi Masaoka[1], Haiyan Zhao[2], Danielle R. Hirsch[3,4], Michael P. D'Erasmo[3,4], Christine Meck[3,4], Brittany Varnado[2], Joel Baines[5], John A. Beutler[6], John E. Tavis[7], Lynda A. Morrison[7], Ryan P. Murelli[3,4], Liang Tang[2] and Stuart F.J. Le Grice[1]

[1]Basic Research Laboratory, National Cancer Institute, Frederick, MD 21702, USA; [2]Department of Molecular Biosciences, University of Kansas, Lawrence, KS 66045, USA; [3]Department of Chemistry, Brooklyn College, City University of New York, Brooklyn, NY 11210, USA, [4]Department of Chemistry, The Graduate Center, City University of New York, New York, NY, 10016, USA; [5]School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803m USA; [6]Molecular Targets Laboratory, National Cancer Institute, Frederick, MD 21702, USA; [7]Department of Molecular Microbiology, St. Louis University School of Medicine, St. Louis

Vif, APOBECs, and Inhibition

T36. CBF-β Dispensability for Non-Primate Vif Function Hints at HIV-1 Accessibility to Alternative Routes Through Cellular Pathways

Aya Khwaja, Ailie Marx, and Akram Alian
Faculty of Biology, Technion – Israel Institute of Technology, Haifa 320003, Israel

T37. Vif-Specific Fab Inhibits Ubiquitination and Degradation of A3F

Jennifer M. Binning[1], Natalia Sevillano[1], Judd F. Hultquist[2], Nathalie Caretta Cartozo[1], Hai Ta[1], Nevan J. Krogan[2], Charles S. Craik[1], and John D. Gross[1]
[1]Department of Pharmaceutical Chemistry, University of California, San Francisco, California 94158, USA; [2]Department of Cellular and Molecular Pharmacology, University of California-San Francisco, San Francisco, California 94158, USA.

T38. Human Restriction Factor and Genomic DNA Modifier APOBEC3B: NMR Structure, Substrate Binding and Deaminase Activity

In-Ja L. Byeon[1,2], Chang-Hyeock Byeon[1,2], Mithun Mitra[3], Tiyun Wu[3], Dustin Singer[3], Judith G. Levin[3] and Angela M. Gronenborn[1,2]
[1]Pittsburgh Center for HIV Protein Interactions and [2]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260, USA. [3]Section on Viral Gene Regulation, Program on Genomics of Differentiation, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-2780, USA.

T39. Assembly and Characterization of the HIV Vif E3 Ligase with Full-Length Human APOBEC3G

Hai M. Ta, Jennifer M. Binning, Nathalie Caretta Cartoro, Mikhaela Arambulo and John D. Gross
Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158, USA

T40. Structure of the Vif-Binding Domain of the Antiviral Enzyme APOBEC3G

Takahide Kouno[1,2], Elizabeth M. Luengas[3], Megumi Shigematsu[1], Shivender M. D. Shandilya[2], JingYing Zhang[1], Luan Chen[1], Mayuko Hara[1], Celia A. Schiffer[2], Reuben S. Harris[3], and Hiroshi Matsuo[1]
[1]Biochemistry, Molecular Biology and Biophysics Department, Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA; [2]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School Worcester, Massachusetts, USA; [3]Biochemistry, Molecular Biology and Biophysics Department, Masonic Cancer Center, Center for Genome Engineering, Institute for Molecular Virology, University of Minnesota, Minneapolis, Minnesota, USA

T41. Interactions Between APOBEC3 and Viral and Cellular RNA

Ashley York[1,2*], Sebla B Kutluay[3*], Paul D Bieniasz[1,2,4]
*[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; [2]The Rockefeller University, Laboratory of Retrovirology, New York, NY; [3]Washington University School of Medicine in St. Louis, Department of Molecular Microbiology, St. Louis, MO; [4]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, New York, NY; *equal contributions*

T42. Structural and Specificity Studies of APOBEC3 Domains

Tania V. Silvas[1], Shivender M.D. Shandilya[1], Markus-Frederik Bohn[1], Shurong Huo[1], Ellen A. Nalivaika[1], Takahide Kouno[2], Nese Kurt-Yilmaz[1], Mohan Somasundaran[3], Reuben S.Harris[2], Matsuo Hiroshi[2], and Celia A. Schiffer[1]

[1]Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School Worcester, MA 01605. USA; [2]Department of Biochemistry, Molecular Biology and Biophysics, Institute for Molecular Virology, University of Minnesota, Minneapolis, MN 55455. USA; [3]Department of Pediatrics and Program in Molecular Medicine, University of Massachusetts Medical School Worcester, MA 01605. USA

T43. Editing Editors with Editing

Michael A. Carpenter, Amber R. Schoenecker, and Reuben S. Harris

University of Minnesota, Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, Center for Genome Engineering, Masonic Cancer Center, Minneapolis, Minnesota, USA

T44. Progress on Developing Small Chemical Inhibitors of the APOBEC3 Family of DNA Cytosine Deaminases

Ming Li, Margaret E. Olson, Daniel A. Harki, and Reuben S. Harris

University of Minnesota, Department of Biochemistry, Molecular Biology, and Biophysics, Institute for Molecular Virology, Center for Genome Engineering, Masonic Cancer Center, Minneapolis, Minnesota, USA

Integration

BACK

T45. Structural and Functional Characterization of The Mouse Mammary Tumor Virus Intasome

Allison Ballandras-Colas[1], Monica Brown[2], Peter Cherepanov[3], Dmitry Lyumkis[2], Alan Engelman[1]

[1]Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA, USA; [2]Helmsley Center for Genomic Medicine, Salk Institute for Biological Studies, La Jolla, CA, USA; [3]Clare Hall Laboratories, Francis Crick Institute, South Mimms, UK

T46. Modeling Molecular Recognition by HIV-1 Integrase and Protease Using Binding Free Energy Methods

Nanjie Deng[1], Stefano Forli,[2] Ahmet Mantes,[1] James Fuchs,[3] Mamuka Kvaratskhelia,[3] Arthur J. Olson[2], Ronald M. Levy[1]

[1]Center for Biophysics and Computational Biology, Temple University, Philadelphia, PA; [2]The Scripps Research Institute, La Jolla, CA; [3]Ohio State University, Columbus, OH

T47. Molecular Dynamics Studies of ASV and HIV Integrase Reaching Dimers

Sangeetha Balasubramanian[1], Muthukumaran Rajagopalan[1], Ravi Shankar Bojja[2], Anna Marie Skalka[2], Mark D. Andrake[2], and Amutha Ramaswamy[1]

[1]Centre for Bioinformatics, School of Life Sciences, Pondicherry University , R. V. Nagar, Kalapet , Puducherry , 605014 , India; [2]Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

T48. Visualizing HIV-1 Integration

Ryan Stultz and David McDonald
Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, OH

T49. Imaging Studies Demonstrate that Trafficking of HIV-1 Pre-Integration Complexes into the Nucleus Requires TNPO3 and Nuclear CPSF6 Expression

Zhou Zhong[1], Callen Wallace[2], Douglas K. Fischer[1], Christopher Kline[3], Simon C. Watkins[2], and Zandrea Ambrose[1,3]
[1]Molecular Virology and Microbiology Program, [2]Department of Cell Biology and [3]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

T50. Embryonic Lethality Due to Arrested Cardiac Development in Psip1/Hdgfrp2 Double-Deficient Mice

Hao Wang, Ming-Chieh Shun, Amy K. Dickson, and Alan Engelman
Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA 02215

SAMHD1, Vpu, and Nef

BACK

T51. CCL19 Mediates Efficient HIV-1 Infection of Naïve and Central Memory $CD4^+$ T Cells without Alteration of F-Actin Density or dNTP Concentration

Jenn Zerbato[1,2], Gina Lenzi[3], Baek Kim[3], Zandrea Ambrose[1,2], Simon Watkins[1,4] and Nicolas Sluis-Cremer[1,2]
[1]Pittsburgh Center for HIV Protein Interactions; [2]Department of Medicine, Division of Infectious Diseases, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Department of Pediatrics, Emory University School of Medicine, Atlanta, GA; [4]Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA

T52. Microscopic Approach to Investigate Binding Partners of Vpu

Yi-Liang Liu[1], Shujun Yuan[2], Sobha Thamminana[1] and Robert M. Stroud[1]
[1]Department of Biochemistry and Biophysics, University of California, San Francisco, California, USA; [2]Department of Protein Science, Bayer HealthCare, San Francisco, California, USA

T53. HIV-1 Nef Hijacks Clathrin Coats by Stabilizing AP-1 Polygons

Qing-Tao Shen[1], Xuefeng Ren[1], Rui Zhang[1,2], Il-Hyung Lee[1] and James H. Hurley[1,2]
[1]Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences, University of California, Berkeley, Berkeley, CA 94720, [2]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

T54. Probing Conformational States Of HIV-Host Complexes With Recombinant Fabs

Natalia Sevillano[1], Florencia La Greca[1], Hai Ta[1], Jennifer Binning[1], Xuefeng Ren[2], Bei Yang[2], James Hurley[2], John Gross[1] and Charles S. Craik[1].
[1]Department of Pharmaceutical Chemistry, University of California San Francisco, San Francisco, USA; [2]Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA

Screening and Computational Approaches

BACK

T55. The Genetic Interaction Landscape of HIV Infection in Human Cells

David E. Gordon, Ariane Watson, Assen Roguev, Raj Bhatnagar, David C. Crosby, Nevan J. Krogan
Department of Cellular and Molecular Pharmacology, California Institute for Quantitative Biosciences, University of California, San Francisco

T56. Rapid Experimental SAD Phasing and Hot-Spot Identification with Halogenated Fragments

Joseph D. Bauman, Jerry Joe E. K. Harrison, and Eddy Arnold
Center for Advanced Biotechnology and Medicine, Department of Chemistry and Chemical Biology, Rutgers University, 679 Hoes Lane, Piscataway, NJ 08854

T57. Incorporating Property-Based Volume Overlap with DOCK Descriptor Score

Yuchen Zhou[1] and Robert C. Rizzo[1, 2, 3]
[1]Department of Applied Mathematics and Statistics, [2]Institute of Chemical Biology & Drug Discovery, [3]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

T58. Computational Design of Carbohydrate-Binding Proteins with Incorporation of Carbohydrate Flexibility and Mutation

Yiwei Cao[1,2] and David F. Green.[1,2]
[1]Department of Applied Mathematics and Statistics, Stony Brook University, Stony Brook, NY, 11794-3600; [2]Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY

T59. Estimation of the Binding Energies of Drug-like and Nondrug-like Molecules Docked in the Active Site of 1BIS.pdb

Julie B. Ealy, Paolo Flauta, Matthew Mekolichick, Liliana Nassar, and Kelly Youwakim
Penn State Lehigh Valley, 2809 Saucon Valley Road, Center Valley, PA

T60. Protocol Development to Include Solvated Molecular Footprints in Lead Discovery

Jiaye Guo[1] and Robert C. Rizzo[2,3,4]
[1]Graduate Program in Biochemistry and Structural Biology, Stony Brook University, Stony Brook, New York 11794, [2]Department of Applied Mathematics & Statistics, Stony Brook University, Stony Brook, New York 11794, [3]Institute of Chemical Biology & Drug Discovery, Stony Brook University, Stony Brook, New York 11794, [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, New York 11794.

T61. Enrichment of Virtual Screening Results with Interaction Probe Analysis

Daniel N. Santiago, Stefano Forli, Rik Belew, Arthur J. Olson
HIV Interaction and Viral Evolution Center, The Scripps Research Institute

T62. A Genetic Algorithm for DOCK to Aid in *De Novo* Design

Courtney D. Singleton[1], William J. Allen[2], and Robert C. Rizzo[2,3,4]
[1]Department of Pharmacology, [2]Department of Applied Mathematics & Statistics, [3]Institute of Chemical Biology & Drug Discovery, [4]Laufer Center for Physical & Quantitative Biology, Stony Brook University, Stony Brook, NY 11794

Friday Posters

BACK

RNA Splicing

F1. Structural Insights into an HIV Splicing Regulatory Complex

Christopher E. Morgan[1], Jennifer L. Meagher[2], Jeffrey D. Levengood[1], James Delproposto[2], Carrie Rollins[1], Jeanne A. Stuckey[2] and Blanton S. Tolbert[1]
[1]Department of Chemistry, Case Western Reserve University, Cleveland, OH; [2]Life Sciences Institute, University of Michigan, Ann Arbor, MI

F2. Combining PrimerID and Deep Sequencing to Quantify HIV-1 Splicing, Measure Mutation Rates, and Screen Mutations in Splice Control Elements

Ann Emery[1], and Ronald Swanstrom[2]
[1]Genetics and Molecular Biology Curriculum, [2]Department of Biochemistry and Biophysics, University of North Carolina Chapel Hill

F3. Solution Structure of the HIV Intronic Splicing Silencer (ISS)

Niyati Jain, Christopher E. Morgan, Brittany D. Rife and Blanton S. Tolbert
Case Western Reserve University, jxn172@case.edu

F4. Targeting Dynamic Ensembles of Exon Splicing Silencer 3 Domain of HIV-1 Genomic RNA

Heidi S. Alvey[a,d], Shan Yang[a], Dawn Kellogg[a], Bharathwaj Sathyamoorthy[a,d], Laura R. Ganser[a,d], David Case[b,d], Blanton S. Tolbert[c,d] and Hashim M. Al-Hashimi[a,d]
[a]Department of Biochemistry and Chemistry, Duke University, Durham, NC 27710; [b]Department of Chemistry and Chemical Biology and BioPaPS Institute, Rutgers University, Piscataway, NJ 08854; [c]Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106; [d]Center for HIV RNA Studies (CRNA), University of Michigan Medical School • Department of Microbiology and Immunology, Ann Arbor MI 48109.

F5. Examination of The Disordered C-Terminal Domain of a Host Regulator of HIV-1 Splicing

Jeffrey D. Levengood, Christopher Morgan, and Blanton S. Tolbert
Department of Chemistry, Case Western Reserve University, Cleveland, OH

Rev and RNA export

BACK

F6. Mechanism of the DDX1-Assisted Assembly of Rev on the Rev Response Element

Rajan Lamichhane, Ingemar Pedron, John Hammond, James Williamson and David Millar
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037 USA

F7. A Mechanism for DDX1-Mediated Rev Binding to the RRE RNA: An RNA Structure-Driven Process

John Hammond, Rajan Lamichhane, David Millar, and James Williamson
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037 USA

F8. Structural Investigation into the HIV RNA Nuclear Export Complex

Amber M. Smith, David S. Booth, Bhargavi Jayaraman, Yifan Cheng and Alan Frankel
Department of Biochemistry and Biophysics and the HIV Accessary & Regulatory Complexes Center, University of California, San Francisco, San Francisco, CA 94158.

F9. Roles of RNA Helicases DDX1/DDX21 Nuclear Export and Stability of Unspliced HIV-1 mRNA

Hui-Yi Chu[1], John Hammond[2], Li Zhou[2], Ilean Chai[3,4], Souad Naji, Mary Lewinski[5] , Bruce Torbett[4], James R. Williamson[2], Larry Gerace[1]
[1]Depts. of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA; [2]Depts. of Molecular Biology and Depts. of Chemistry, The Scripps Research Institute, La Jolla, CA; [3]UCSD Biomedical Sciences Program, University of California, San Diego, La Jolla, CA; [4]Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; [5]Division of Infectious Diseases, University of California, San Diego, La Jolla, CA

RNA Structure, Function, and Packaging

BACK

F10. Parallel High Throughput Experimental and Computational Screening Against a Flexible HIV RNA Target

Laura R. Ganser[1,4], Janghyun Lee[2], Bharathwaj Sathyamoorthy[1,4], Aman Kansal[1], Raymond Li[1], Paul Bieniasz[3,4], Hashim M. Al-Hashimi[1,4]
[1]Department of Biochemistry, Duke University, Durham, NC 27710; [2]Department.of Chemistry, University of Michigan, Ann Arbor, MI 48109; [3]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY 10016; Laboratory of Retrovirology and Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10016; [4]Center for HIV RNA Studies (CRNA), University of Michigan Medical School • Department of Microbiology and Immunology, Ann Arbor MI 48109-5620

F11. Expansion of a Novel RNA-Binding Scaffold to Target HIV-1 *TAR* RNA

Patwardhan, N.N.[1], Ganser, L.[2], Kapral G. J. [1], Eubanks, C.S. [1], Lee, J. [2], Sathyamoorthy B. [2], Al-Hashimi, H.M.[2], and Hargrove, A.E.[1,2]
[1]Department of Chemistry, Duke University, Durham, NC; [2]Department of Biochemistry, Duke University School of Medicine, Durham, NC

F12. Novel *Cis*-Acting RNA Elements in the HIV-1 Genome

Matthew A Takata[1,2], Steven J Soll[1,2,3], Daniel Blanco-Melo[1,2], Paul D Bieniasz[1,2,3]
[1]Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY; [2]The Rockefeller University, Laboratory of Retrovirology, New York, NY; [3]Howard Hughes Medical Institute, Aaron Diamond AIDS Research Center, New York, NY

F13. Data Mining for RNA Chemical Shift Predictions

Joshua D. Brown[1], Michael F. Summers[1], and Bruce A. Johnson[2]
[1]Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland; Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250; [2]One Moon Scientific, Inc, 839 Grant Ave., Westfield, NJ 07090

F14. Role of HIV-1 Genomic RNA Structure in Human Lysyl-tRNA Synthetase Recruitment

William A. Cantara, Roopa Comandur, Joshua Hatterschide, Erik D. Olson, and Karin Musier-Forsyth
Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retrovirus Research, The Ohio State University, Columbus, OH, USA

F15. Modifications of Nucleic Acid Bases on $tRNA^{Lys3}$ Affect Interactions with the Matrix Domain of the HIV-1 Gag Polyprotein

Christy R. Gaines, Tarik D. Hawkins, Emre B. Tkacik, and Michael F. Summers
HHMI at University of Maryland, Baltimore County

F16. RNA Helicase A is Co-Packaged with HIV-1 RNA via Direct Interactions with the 5'-Leader RNA

Zhenwei Song[1], Ioana Boreas[2], Kathleen Boris-Lawrie[2], and Xiao Heng[1]
[1]Department of Biochemistry, University of Missouri, Columbia, MO, 65211; [2]Department of Veterinary Biosciences, Center for Retrovirus Research, The Ohio State University, Columbus, OH

F17. Co-Transcriptional Packaging of Retroviral Genomic RNA by the Gag Polyprotein

Rebecca J. Kaddis[1], Eunice Chen[1], Breanna L. Rice[1], Estelle Chiari-Fort[1], Kevin Tuffy[1], Matthew Stake[1], Nikoloz Shkriabai[2], Mamuka Kvaratskhelia[2], Alan Cochrane[3], and Leslie J. Parent[1]
[1]Department of Medicine, Penn State College of Medicine, Hershey, PA 17033; [2]College of Pharmacy, The Ohio State University, Columbus, OH; [3]Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

F18. How Does HIV-1 mRNA Initiate Viral Protein Synthesis Independently of eIF4F?

Sarah Fritz and Kathleen Boris-Lawrie
Department of Veterinary Biosciences, Center for Retrovirus Research, Center for RNA Biology, The Ohio State University, Columbus Ohio, USA

F19. Structural Isoforms of HIV-1 5' Untranslated Region Expose Distinct Protein Binding Sites that Promote Utilization as mRNA or Genomic RNA

Ioana Boeras[1], Zhenwei Song[2], Xiao Heng[2] and Kathleen Boris-Lawrie[1]
[1]Department of Veterinary Biosciences, Center for Retrovirus Research, Center for RNA Biology, The Ohio State University, Columbus Ohio; [2]Department of Biochemistry, University of Missouri, Columbia, MO

F20. Understanding HIV-1 Packaging Signal by Single-Molecule Spectroscopy

Mauricio Comas-Garcia[1], Siddhartha A.K. Datta[1], Rajat Varma[2] and Alan Rein[1]
[1]Retroviral Assembly Section, HIV Drug Resistance Program, National Cancer Institute, Frederick, MD 21702, USA; 2Laboratory of Systems Biology, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892, USA

F21. Characterization of HIV-1 5'-Leader Dynamics using NMR Relaxation Measurements

Jan Marchant[1], Sarah Keane[1], Sarah Monti[1], Jinfa Ying[2], Ad Bax[2], and Michael Summers[1]
[1]Howard Hughes Medical Institute at UMBC and [2]Laboratory of Chemical Physics, NIDDK, NIH

F22. Characterization of Monomer/Dimer Equilibrium of the HIV-1 5'-Leader with Variable +1 mRNA Start Sites and Presence of the 5'-Methylguanosine Cap

Sarah Monti, Verna Van, Nicholas Bolden, Joshua Brown, and Michael Summers
HHMI, University of Maryland, Baltimore County, USA

F23. Direct Probing of the Dimer Interface in the 688-Nucleotide HIV-1 Leader RNA

Sarah C. Keane, Xiao Heng, Jan Marchant, Gregory Carter, Justin Santos, Alyssa Florwick, Heather Frank, Sayo McCowin, and Michael F. Summers
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

F24. Characterizing RNA Dimerization Mechanisms of the Genomic 5´-Leader in Human and Simian Immunodeficiency Viruses

Thao Tran, Michelle Seu, Jessica Zaki, Ally Yang, and Michael Summers
Department of Chemistry and Biochemistry, University of Maryland, Baltimore County

F25. Characterization of HIV-1 Gag-gRNA Interactions

Erik D Olson[1], William A Cantara[1], Brian R Thompson[1], Devrishi Goswami[2], Monica Brown[3], Ioulia Rouzina[4], Dmitry Lyumkis[3], Patrick R Griffin[2], and Karin Musier-Forsyth[1]
[1]Department of Chemistry and Biochemistry, Center for RNA Biology, and Center for Retroviral Research, The Ohio State University, Columbus, OH; [2]Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL; [3]Helmsley Center for Genomic Medicine, Salk Institute for Biological Studies, La Jolla, CA; [4]Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN

Gag and Env Trafficking and Assembly

BACK

F26. Structural and Functional Insights into the Binding of Human TSG101 with a Small Molecule HIV-1 Budding Inhibitor

Madeleine Strickland[1], Lorna S. Ehrlich[2], Mahfuz Khan[3], Michael D. Powell[3], Carol Carter[2], and Nico Tjandra[1]
[1]NHLBI, NIH, Bethesda, MD; [2]Department of Molecular Genetics & Microbiology, School of Medicine, Stony Brook University, Stony Brook, NY; [3]Department of Microbiology, Biochemistry, and Immunology, Morehouse School of Medicine, Atlanta, GA

F27. Angiomotin Functions in HIV-1 Assembly and Budding

Steven L Alam, Gaelle Mercenne, Tuscan Thompson, Jun Arii, Matthew S Lalonde, Wesley I Sundquist
Department of Biochemistry, University of Utah, Salt Lake City, United States

F28. Identification of a Novel Structural Element in Gag Important for the Assembly, Release and Maturation of HIV-1 Particles

Mariia Novikova[1], Muthukumar Balasubramaniam[1], Sagar Kudchodkar[1], Ferri Soheilian[2], and Eric O. Freed[1]

[1]Virus-Cell Interaction Section, HIV Dynamics and Replication Program, NCI-Frederick, Frederick, MD; [2]Electron Microscope Laboratory, Cancer Research Technology Program, Leidos Biomedical Research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

F29. The Matrix Trimer Is an Essential Structural Component of the Infectious HIV-1 Particle

Philip R. Tedbury, Mariia V. Novikova, Sherimay D. Ablan and Eric O. Freed

HIV Dynamics and Replication Program, NCI at Frederick, Frederick, MD. USA.

F30. *In vivo SELEX* Reveals Novel Protein Determinants for Murine Leukemia Virus (MLV) Envelope Glycoprotein Functionality

Daniel J. Salamango[1], Khalid K. Alam[1], Donald H. Burke[1, 2], and Marc C. Johnson[2]

Departments of Biochemistry[1] and Molecular Microbiology and Immunology[2], University of Missouri-Columbia

F31. HERV-K Env Can Interfere with HIV-1 Infection

Sandra Terry[1], Daria Brinzevich[1], Teddy John Wohlbold[1], Gene Tan[1], Juan Ayllon[1,2], Adolfo Garcia-Sastre[1,2], Florian Krammer[1], Viviana Simon[1,2], Lubbertus C.F. Mulder[1,2]

[1]Department of Microbiology, [2]Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New York, NY

F32. A Tyrosine-Based Motif in the HIV-1 Envelope Glycoprotein Tail Mediates Cell Type- and Rab11-FIP1C-Dependent Incorporation into Virions

Mingli Qi[1], Hin Chu[2], Xuemin Chen[1], Junghwa Choi[1], Xiaoyun Wen[1], Jason Hammonds[1], Lingmei Ding[1], Eric Hunter[3], and Paul Spearman[1]

[1]Department of Pediatrics, Emory University and Children's Healthcare of Atlanta, Atlanta, Georgia; [2]Department of Microbiology, Queen Mary Hospital, University of Hong Kong, Hong Kong Special Administrative Region, China; [3]Emory Vaccine Center, Yerkes National Primate Research Center, Emory University, Atlanta, GA 30322

Gag/Membrane Interaction and Budding

BACK

F33. Purified RSV Gag is Extended and Flexible and Binds to Membranes in a Cooperative Fashion that Depends on the SP Domain.

Robert Dick[1], Siddhartha Datta[2], Hirsh Nanda[3], Marilia Barros[4], Xianyang Fang[5], Yun-Xing Wang[5], Mathias Lösche[4], Alan Rein[2], and Volker Vogt[1]

[1]Dept of Molecular Biology and Genetics, Cornell University; [2]HIV Drug Resistance Program, National Cancer Institute; [3]NIST Center for Neutron Research, National Institute of Standards and Technology; [4]Dept of Physics and Biological Physics, Carnegie Mellon University; [5]Structural Biophysics Laboratory, National Cancer Institute

F34. Effect of Membrane Order and Charge on the Binding of Retroviral Gag Proteins to Membranes In Vitro

Yi Wen, Robert A. Dick, Gerald W. Feigenson and Volker M. Vogt
Dept. of Molecular Biology and Genetics, Cornell University, Ithaca NY 14853

F35. Line Tension and Bending Modulus for Models of the Cell Plasma Membrane

Rebecca D. Simpson, Sanjula P. Wickramasinghe, David G. Ackerman, Mary B. Kim, and Gerald W. Feigenson
Department of Molecular Biology & Genetics, Cornell University, Ithaca, New York

F36. Membrane Composition and Protein Lipidation as Determinants of the Free Energy of HIV-1 Matrix Membrane Binding

Marilia Barros, Frank Heinrich, Hirsh Nanda, Siddartha A. K. Datta, Alan Rein, and Mathias Lösche

F37. Investigation of Feline Immunodeficiency Virus Matrix Protein Assembly to the Plasma Membrane

Janae L. Baptiste, Morgan B. Moser, Colin T. O'Hearn, and Michael F. Summers
Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

F38. Modes of Interaction of Retroviral Proteins with Lipid Bilayers: A Molecular Dynamics Simulations Study

Milka Doktorova, Rob Dick, Harel Weinstein, Fred Heberle, Volker Vogt, and Gerald Feigenson
Weill Cornell Medical College, Cornell University

F39. Three-Dimensional Structural Characterization of HIV-1 Tethered to Human Cells by Cryo-electron Tomography

Joshua D. Strauss[1], Jason E. Hammonds[1], Hong Yi[2], Lingmei Ding[1], Paul W. Spearman[1], and Elizabeth R. Wright[1,2].
[1]Department of Pediatrics, Emory University School of Medicine, and Children's Healthcare of Atlanta. Atlanta, GA 30322; [2]Robert P. Apkarian Integrated Electron Microscopy Core. Emory University. Atlanta, GA 30322.

F40. Cellular Release and Uptake of Designed Enveloped Nanoparticles

Jörg Votteler[1], Sue Yi[2], Yang Hsia[2], Jacob Bale[2], David Baker[2], Neil King[2], and Wes Sundquist[1]
[1]Department of Biochemistry, University of Utah, Salt Lake City, Utah 84113; [2]Institute for Protein Design, University of Washington, Seattle, Washington 98195

Maturation and Inhibition

BACK

F41. Studies of HIV-1 Maturation *in vitro*

Jiying Ning[1], Xiaofeng Fu[1], Ernest L. Yufenyuy[2], Christopher Aiken[2], and Peijun Zhang[1]
[1]Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA; [2]Department of Pathology, Microbiology and Immunology, Vanderbilt School of Medicine, Nashville, TN 37232, USA

F42. MAS NMR Characterization of HIV-1 Maturation Intermediates

Caitlin M. Quinn[1,2], Christopher L. Suiter[1,2], Mingzhang Wang[1,2], Jinwoo Ahn[2,3], Sherimay Ablan[4], Eric O. Freed[4], Luigi J. Alvarado[5,6], Owen Becette[5,6], T. Kwaku Dayie[5,6], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

[1]University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716; [2]Pittsburgh Center for HIV Protein Interactions, University of Pittsburgh, Pittsburgh, PA 15260; [3]Department of Structural Biology, University of Pittsburgh School of Medicine, 3501 Fifth Ave., Pittsburgh, PA 15260; [4]HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Building 535, Room 110, Frederick, MD 21702; [5]University of Maryland, Department of Chemistry and Biochemistry, College Park, MD 20742; [6]The Center for HIV RNA Studies, University of Michigan Medical School, Ann Arbor, MI 48109

F43. Development of Potent and Broadly Active "Second-Generation" HIV-1 Maturation Inhibitors; Insights into Gag Structure and Function

Emiko Urano[1], Sherimay Ablan[1], Rebecca Mandt[1], David E. Martin[2], Ritu Gaur[3], T. J. Nitz[2], Carl T. Wild[*2], and Eric O. Freed[1]

[1]HIV Dynamics and Replication Program, National Cancer Institute, Frederick, MD; [2]DFH Pharma, Inc., Gaithersburg, MD; [3]South Asian University, New Delhi, India

F44. Development of Novel HIV Maturation Inhibitors against HIV Subtype C

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PR and Inhibition

BACK

F45. Exploring the Interdependence of Sub-Sites in the Active Site of HIV-1 Protease

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F46. Construction of a SHIV Theoretically Capable of Modeling HIV-1 Protease Inhibitor Potency in Non-Human Primates

Jesse Crayle[1], Sook-Kyung Lee[1], Celia Schiffer[2], and Ronald Swanstrom[1]

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F47. Hydration and Dynamics in Inhibitor-Bound HIV-1 Protease using NMR Spectroscopy

John D. Persons[1], Shahid N. Khan[1], Michel Guerrero[1], Akbar Ali[2], Celia A. Schiffer[2] and Rieko Ishima[1]

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F48. Exploring Surface Sites on HIV Protease as Targets for Inhibitors: Promises and Failures

Tiefenbrunn, T.[1], Forli, S.[1], Happer, M.[2], Gonzalez, A[3]., Baksh, M. M.[4], Chang, M. W.[5], Tsai, Y.-S.[3], Lin, Y.-C.[2], Perryman, A. L.[1], Rhee, J.-K.[4], De Vera, I.[6], Kojetin, D.[6], Torbett, B.E.[5], Olson, A. J.[1], Soltis, M.[3], Elder, J. H.[2], Finn, M. G.[4], Stout, C. D.[1]

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F49. Implications of Hinge Region Natural Polymorphisms on HIV-1 PR Structure, Dynamics and Drug-Pressure Selected Evolution

Zhanglong Liu[1], Xi Huang[1], Lingna Hu[1], Linh Pham[1], Katye Poole[2], Yan Tang[2], Brian P. Mahon[2], Wenxing Tang[2], Kunhua Li[1], Nathan E. Goldfarb[2], Ben M. Dunn[2], Robert McKenna[2], Gail E. Fanucci[1*]

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F50. Mapping The Fitness Landscapes of Drug-Resistance in HIV Protease

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F51. Analyses of Accessory Mutations in HIV-1 Protease Reveal Interdependent Mechanisms of Resistance

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F52. Understanding the Dynamics of Drug Resistance Mutations using HIV-1 Protease as a Model System

Janet Paulsen[1], Florian Leidner[1], Salma Rafi[2], Akbar Ali[1], Sook-Kyung Lee[3], Ronald Swanstrom[3], Konstantin Zeldovich[1], Woody Sherman[2], Celia Schiffer[1]

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F53. Selection to Confirm Novel Resistance Pathways to Potent New HIV-1 Protease Inhibitor

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F54. Understanding Antiviral Drug Resistance and Broadly Neutralizing Antibodies in Influenza

Kristina Prachanronarong, Zhen Zhang, Ellen Nalivaika, Aysegul Ozen, Safak Yilmaz, Kelly Thayer, Konstantin Zeldovich, Jennifer Wang, Wayne Marasco, Robert Finberg, and Celia Schiffer
University of Massachusetts Medical School, Worcester, MA, USA

F55. Structural and Thermodynamic Investigation of the Role of Macrocyclization in Hepatitis C Virus Protease Inhibitor MK-5172's Potency and Resistance Profile

Djadé I. Soumana[1], Akbar Ali[1], Kristina L. Prachanronarong[1], Cihan Aydin[1] and Celia A. Schiffer[1]

NC/RNA Interaction and Inhibition

BACK

F56. Exploring Tryptophan Stacking Capabilities in the HIV-1 Nucleocapsid Protein: Insights From Docking Calculations

Victor H.F. Bernardes[1,2], Raphael E.F. de Paiva,[1,3], Maria D. Vargas[2], Pedro P. Corbi[3], and Nicholas P. Farrell[1]
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F57. HIV-1 Nucleocapsid-RNA Antagonists Based on Zn-Ejecting Metal Complexes

Raphael E. F. de Paiva[1,2], Camilla Abbehausen[1], Sarah Spell[2], Samantha Tsotsoros[2], Erica J. Peterson[2], John B. Mangrum[3], Daniele Fabris[3] and Nicholas P. Farrell[2]
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F58. Predicting Metalation Sites on HIV-1 Nucleocapsid Protein: A Bioinformatics Approach Using Blue Star Sting

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CA and Core Assembly

BACK

F59. High Resolution Structure of HIV-1 Capsid Assembly

Gongpu Zhao[1,2], Chuang Liu[1,2], Xueming Li[3,4], Jiying Ning[1,2], Shenping Wu[3], Randall T. Schirra Jr.[1,2], Angela M. Gronenborn[1,2], Yifan Cheng[3] and Peijun Zhang[1,2]
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F60. Structural Studies of HIV-1 Capsid Protein Assemblies by Sensitivity Enhanced Magic Angle Spinning NMR

Rupal Gupta[1,2], Manman Lu[1,2], Guangjin Hou[1,2], Marc Caporini[4], Melanie Rosay[4], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,2]

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F61. Coarse-Grained Simulation Reveals Key Features of HIV-1 Capsid Self-Assembly

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F62. Structural Studies of HIV-1 Capsid CA Protein Assemblies: Insights into Structure by Magic Angle Spinning NMR Spectroscopy and Interactions with TRIM5α by Magic Angle Spinning NMR

Mingzhang Wang[1,2], Caitlin M. Quinn[1,2], Guangjin Hou[1,2], Huilan Zhang[1,2], Christopher L. Suiter[1,2], Manman Lu[1,2], Jinwoo Ahn[2,3], In-Ja L. Byeon[2,3], Christopher R. Aiken[2,4], Angela M. Gronenborn[2,3], Tatyana Polenova[1,2]

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Viral Evolution

BACK

F63. Structural Basis and Distal Effects of Gag Substrate Co-Evolution in Drug Resistance to HIV-1 Protease

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F64. Identifying Patterns of Correlated Resistance Mutations in HIV-1

William F. Flynn[1,2], Allan Haldane[2,3], and Ronald M. Levy[2,3]

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F65. Co-Evolution of HIV-1 Protease and p1-p6 Modulates Gag Processing

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F66. Viral Quasispecies and Mutational Analysis using Next-Generation Sequencing

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F67. Impact of the HLA B*57 Allele on Intra-Host HIV-1 Capsid Phylodynamics and RNA Secondary Structure Diversity

Brittany D. Rife[1,2], Gary B. Fogel[3], Susanna L. Lamers[4], David J. Nolan[1,2], Melissa Norstrom[5], Frederick M. Hecht[6], Annika C. Karlsson[5], Marco Salemi[1,2]
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F68. Deep Mutational Scanning of the Overlapped HIV-1 Genes *Tat* and *Rev* Reveals a Selfish Segregation of Functional Motifs

Jason D. Fernandes[1,2], Tyler B. Faust[1,3], Nicolas Strauli[4,5], Cynthia Smith[1], David C. Crosby[1], Ryan D. Hernandez[4], and Alan D. Frankel[1]
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F69. Combining Chemical and Intra-Host Evolutionary Information for HIV-1 RNA Secondary Structure Prediction

Brittany D. Rife[1,2], Gary B. Fogel[3], Enoch Liu,[3] Susanna L. Lamers[4], David J. Nolan[1,2], Melissa Norstrom[5], Frederick M. Hecht[6], Annika C. Karlsson[5], and Marco Salemi[1,2]
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Cellular Release and Uptake of Designed Enveloped Nanoparticles

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Viruses like HIV-1 can assemble and bud from producer cells and deliver cargoes into the cytoplasms of new target cells. In principle, these activities have potential therapeutic applications but current synthetic technologies are often limited by inefficiency and lack of flexibility, whereas viral vectors can be limited by safety concerns, immunogenicity, and difficulties in packaging non-nucleic acid cargoes. As a test of our understanding of the requirements for viral particle assembly, envelopment, budding, and target cell re-entry, we have created designed nanoparticles that spontaneously assemble into different platonic solids, including dodecahedra and icosahedra. These self-assembling proteins were expressed in human cells and used to test the requirements for particle egress and entry.

Designed nanoparticles could be targeted to membranes through the addition of the HIV-1 MA myristoylation signal and other membrane targeting domains. Membrane-associated nanoparticles could be released from cells through the addition of the $p6^{Gag}$ region of HIV-1, and particle release was dependent upon the presence of both the TSG101-binding PTAP and ALIX-binding YPXL motifs, demonstrating that particle release required recruitment of the cellular ESCRT machinery. EM analyses revealed that assembled articles were released predominantly within enveloped extracellular vesicles, rather than as individual particles. A Vpr-β-lactamase fusion protein could be specifically incorporated via a non interaction between the Vpr and $p6^{Gag}$ proteins, and expression of VSV-G protein allowed the particles to enter new target cells, as assayed by β -lactamase activity. Thus, we have designed icosahedral nanoparticles that can self assemble in mammalian cells, bind membranes, exit cells in an ESCRT-dependent fashion, and enter new target cells to deliver enzymatic cargoes. This system will be used as a starting point for optimization of synthetic delivery systems based on the principles employed by enveloped and non-enveloped viruses.

CryoEM Structure of a T=4 Icosahedral HIV-1 Capsid-Like Shell

Mark Yeager[1], Jonathan Wagner[1], Kaneil Zadrozny[1], Banumathi Sankaran[2], Kelly Dryden[1], Barbie K. Ganser-Pornillos[1], and Owen Pornillos[1]

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Interaction of the rotamase cyclophilin A (CypA) with the CA domain of Gag underlies important aspects of HIV-1 replication. Recent data indicate that although CypA is recruited during virion formation, the critical function of CypA is in target cells, where it is proposed to modulate the integrity of the post-entry capsid, enable timely reverse transcription, and shield the genome from pattern recognition receptors that might otherwise mount an interferon response. The kinetics, affinity, and structural details of CA-CypA complexes are well understood from studies of soluble proteins/domains, but biochemical and structural experiments with capsid assemblies can be difficult because of the high dissociation rate between CA and CypA. We therefore employed a protein fusion strategy to design a non-dissociable CA-CypA complex to facilitate such studies. The fusion was designed and optimized using the CA_{NTD}-CypA co-crystal structure as a guide. X-ray structures of the designed CA_{NTD}-CypA fusion confirmed the proper folds of both domains and a similar orientation of the CypA molecule with respect to the CA molecule as in the non-covalent complexes. Furthermore, assembly studies of the full-length R18L CA-CypA protein revealed that particles with T=4 icosahedral symmetry formed in the presence of high concentrations of citrate. By the use of cryoEM and icosahedral image processing, we recently determined a map at 9.6 Å resolution. 3D reconstructions revealed that both the CA lattice and the surrounding CypA layer are well ordered. In addition, the highly α-helical CA protein can be fitted unambiguously into the map to reveal the first subnanometer resolution structure of a mature retroviral capsid-like particle that contains both pentamers and hexamers.

BACK

Retrovirus Dissemination Depends on CD169/Siglec-1 Mediated *Trans*-Infection of Permissive Lymphocytes

Xaver Sewald[1], Mark S. Ladinsky[2#], Pradeep D. Uchil[1#], Jagadish Beloor[3], Ruoxi Pi[1], Thomas T. Murooka[4], Christin Herrmann[1], Nasim Motamedi[1], Michael A. Brehm[5], Dale L. Greiner[5], Leonard D. Shultz[6], Thorsten R. Mempel[4], Pamela J. Bjorkman[2], Priti Kumar[3], and Walther Mothes[1]

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Dendritic cells can capture and transfer retroviruses *in vitro* across synaptic cell-cell contacts to uninfected cells, a process called *trans*-infection. Whether *trans*-infection contributes to retroviral spread *in vivo* remains unknown. Here we visualize the dissemination of retroviruses in secondary lymphoid tissues of living mice. We demonstrate that murine leukemia virus (MLV) and human immunodeficiency virus (HIV) are first captured by sinus-lining macrophages. Virus capture is mediated by CD169/Siglec-1 that recognizes viral gangliosides. MLV-laden macrophages then form long-lived synaptic contacts to *trans*-infect B-1 cells. Infected B-1 cells subsequently migrate to the inter-follicular space to spread the infection through virological synapses. Efficient infection of mice requires CD169, suggesting that retroviruses utilize a combination of fluid-based movement followed by CD169-dependent *trans*-infection to spread *in vivo*.

Progress update from the Pittsburgh Center for HIV Protein Interactions

Angela M. Gronenborn

The Pittsburgh Center for HIV-Protein Interactions (PCHPI) is focused on understanding the interaction of host proteins with HIV-1 during the so-called early stages of HIV-1 infection, those that occur after viral membrane fusion and prior to integration. During the past year, PCHPI members made progress toward understanding the structure and function of MxB (Fribourgh *et al*, Cell Host Microbe 16:627) and carried out structural characterization of the RT p66 homodimer (Sharaf *et al*, Proteins 82:2343) and of APOBEC3H (Mitra *et al*, Retrovirology 12:3). The center has continued its effort to understand the regulation of SAMHD1 (Yan *et al*, J Biol Chem 290:13279; Koharudin et al, J Biol Chem 289:32617) as well as trafficking of the HIV-1 pre-integration complex to the nucleus. Finally, we continue to develop solid-state NMR methodologies for analyzing assembled Capsid and its complexes with TRIM5α and Cyclophilin A (Lu *et al*, J Biomol NMR 61:7; Hou *et al*, J Chem Phys 141:104202). I will briefly summarize the major findings from our studies during the past year, highlighting our structural analysis of Vpx in complex with DCAF1 and SAMHD1.

BACK

Structure of Mature HIV-1 Capsid and Its Interaction with Cyclophilin A

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The mature HIV-1 capsid plays a major role in regulating the early stages of HIV-1 replication by interacting with many host factors, including cyclophilin A (CypA). The binding of CypA to the viral capsid is important for infection, yet the mechanism by which CypA regulates HIV-1 transduction efficiency is unknown. Furthermore, several other host cell proteins, including TRIM-Cyp and NUP358, contain a CypA domain that interacts with viral capsid and regulates viral infectivity. We previously determined the CA tubular assembly structure to 8 Å, using cryoEM, and built an all-atom model of the complete capsid by large scale molecular dynamics simulations. However, the precise chemical environment essential for capsid assembly and how CypA interacts and stabilizes the viral capsid remains unclear. We have now independently reconstructed three cryoEM density maps of CA tubular assemblies from (-12, 11), (-13, 12) and (-13, 11) helical symmetries at 4.5, 5.6 and 5.6 Å resolution, respectively. The 4.5 Å structure clearly resolves bulky side chain densities, helix grooves and connecting loops, enabling accurate molecular modeling at an atomic level. Furthermore, we determined the cryoEM structure of CypA in complex with HIV-1 capsid assembly at 8 Å resolution. The density map unexpectedly displays a distinct, non-random CypA binding pattern in which CypA bridges two adjacent CA hexamers and wraps selectively along the curved CA array. CryoEM structure-based modeling and large scale all-atom molecular dynamics simulations surprisingly reveal that the unique CypA pattern is achieved through an additional, uncharacterized, novel interface such that a single CypA molecule simultaneously interacts with two CA molecules. Solid-state NMR and mutagenesis studies further confirmed that the residues at the novel interface are critical for the interaction and viral infectivity. Our hybrid cryoEM, computational and solid-state NMR studies provide new mechanistic insights into the functional role of CypA in modulating capsid uncoating and viral infectivity.

BACK

Structural Basis for Retroviral Integration into Nucleosomes

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Retroviral integration is catalyzed by a tetramer of integrase assembled on viral DNA ends in a stable complex, known as the intasome. How the intasome interfaces with chromosomal DNA, which exists in the form of nucleosomal arrays, is currently unknown. We show that the prototype foamy virus intasome is proficient at stable capture of nucleosomes as targets for integration. Single-particle cryo-electron microscopy reveals a multivalent intasome-nucleosome interface involving both turns of nucleosomal DNA and one H2A-H2B heterodimer. While the histone octamer remains intact, the DNA is lifted from the surface of the H2A-H2B heterodimer to allow integration at preferred sites, which are offset from the nucleosomal dyad by 3.5 turns of the DNA helix. Mutations disrupting these contacts impinge on the ability of the intasome to engage nucleosomes in vitro and instigate re-distribution of viral integration sites on the genomic scale. Our findings elucidate the molecular basis for nucleosome capture by the viral DNA recombination machinery and underlying nucleosome plasticity that allows integration.

BACK

HIVE Center: HIV Interactions and Viral Evolution of Drug Resistance

Eddy Arnold[1], John Elder[2], Alan Engelman[3], M.G. Finn[4], Valery Fokin[5], Patrick Griffin[6], Stephen Hughes[7], Roger Jones[1], Mamuka, Kvaratskhelia[8], Ron Levy[1], Joe Marcotrigiano[1], Arthur Olson[9], Michael Parniak[10], Alan Rein[7], Dave Stout[9], and Bruce Torbett[11]

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The HIVE Center is focused on the structural and dynamic interactions of the major HIV enzymes, reverse transcriptase/RNAse H, protease and integrase, with their molecular partners and effectors in key processes of the viral life cycle. By studying how the structures of the HIV polyprotein precursors direct assembly, maturation and replication, as well as how HIV-Host interactions drive DNA replication and integration, we explore how therapeutic targeting impacts the evolution of drug resistance and what the structural and dynamic consequences of resistance mutations are on the HIV life cycle. This approach is significant because of the promise of new structural insights into the interdependence of viral mechanisms and the potential for new drug design methodologies and therapeutic strategies.

This overview will present the nature of the collaborations and research within the HIVE Center, with selected examples of work that highlight the connections between experimental and computational approaches to understanding HIV structures and the evolution of drug resistance.

BACK

Unexpected Connections of tRNA Synthetases to HIV-1 Replication: Implications for tRNA Primer Recruitment and Initiation of Reverse Transcription

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All retroviruses use specific host cell tRNAs to prime reverse transcription of their retroviral RNA genomes into DNA. The primer for reverse transcription in HIV-1, human $tRNA^{Lys}$, is selectively incorporated into virions during viral assembly. We previously reported that a specific $tRNA^{Lys}$ binding protein, human lysyl-tRNA synthetase (LysRS), is also specifically packaged into HIV-1 leading to the enrichment of $tRNA^{Lys}$ in virions. Cytoplasmic LysRS is normally localized to a dynamic mammalian multisynthetase complex (MSC). In addition to their well-known physiological function in translation, many tRNA synthetases are mobilized from the MSC and to function in a wide variety of non-translational pathways. Using immunofluorescence and confocal microscopy we find that LysRS localization is dramatically altered upon HIV-1 infection. In uninfected cells, the majority of LysRS is in the MSC, as expected, whereas LysRS is released from the MSC and traffics to the nucleus following HIV-1 infection, suggesting a potential new role of LysRS in HIV-1 replication.

The $tRNA^{Lys}$ primer must be released from the LysRS/$tRNA^{Lys}$ complex prior to annealing onto the primer binding site located in the 5'-UTR, a highly conserved region of the HIV-1 RNA genome responsible for regulating many steps of the retroviral lifecycle. We show that part of this region mimics the L-shaped fold of tRNA and binds LysRS with high affinity, providing a structural basis for understanding how this genomic RNA coordinates interactions with a tRNA-binding host factor to facilitate initiation of reverse transcription.

BACK

Structural and Functional Characterization of the Mouse Mammary Tumor Virus Intasome

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Integrase (IN) is critical for HIV-1 replication, and IN strand transfer inhibitors (INSTIs) comprise 4 of the 5 HAART formulations recommended for the treatment of drug naïve patients. Understanding the mechanisms of drug action and drug resistance is facilitated by the 3-dimensional structures of the drug target. The INSTI target is the intasome – the nucleoprotein complex composed of an IN multimer and the two ends of viral DNA. To date high resolution structures are available only for the prototype foamy virus (PFV) intasome. PFV intasome structural biology advances were in large part due to highly soluble IN protein and the ability to efficiently integrate two viral DNA ends *in vitro*. PFV nevertheless represents just one genus of Retroviridae. Additional intasome structures will accordingly increase the breadth of knowledge of retroviral integration.

We have discovered that mouse mammary tumor virus (MMTV) IN supports the formation of active intasomes *in vitro*. Complexes assembled by differential salt dialysis were purified by gel filtration chromatography for downstream analyses. Intasome-mediated concerted viral DNA integration yielded a 6 bp duplication of target DNA, which is known to occur during MMTV infection. Single-particle cryo-electron microscopy yielded an initial 6.8 Å resolution map for the protein-DNA complex. MMTV IN is composed of the three canonical IN domains – the N-terminal domain (NTD), catalytic core domain (CCD), and C-terminal domain (CTD) – and homology models of the different domains were built using the structures of Rous sarcoma virus IN CCD and CTD and HIV-2 IN NTD as templates. Placement of the domains into the EM map by rigid body docking indicated that the MMTV intasome is composed of an octamer of IN – four dimers of full-length IN – in complex with two viral DNA ends.

The novel octameric MMTV intasome structure was compared with the known tetrameric IN architecture of the PFV intasome, which revealed a common intasome core structure composed of four IN molecules and two viral DNA ends. We propose that the length of the linker that connects the CCD to the CTD, which is relatively short for MMTV IN, is the main factor that dictates the stoichiometry of IN-to-DNA among different retroviral intasomes.

BACK

HIV-1 Reverse Transcriptase Complexed with a Novel 38-mer Hairpin Template-Primer DNA Aptamer: Platform for Structural and Mechanistic Investigations

Eddy Arnold[1], Joseph D. Bauman[1], Kalyan Das[1], Jeffrey DeStefano[2], Irani Ferreira-Bravo[2], Jerry Joe Harrison[1], Sergio E. Martinez[1], Matthew T. Miller[1], Francesc Xavier Ruiz[1], and Steven Tuske[1]

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The development of a modified DNA aptamer that binds HIV-1 reverse transcriptase (RT) with ultra-high affinity has enabled the X-ray structure determination of an HIV-1 RT-DNA polymerase complex to 2.3 Å resolution without the need for an antibody Fab fragment or RT-DNA cross-linking. The 38-mer hairpin-DNA aptamer has a 15 base-pair duplex, a three-deoxythymidine hairpin loop, and a five-nucleotide 5'-template overhang. The aptamer binds RT in a template-primer configuration with the 3'-end positioned at the polymerase active site and has 2'-O-methyl modifications at the second and fourth duplex template positions that interact with amino acid residues in the p66 fingers and palm subdomains. This structure represents the highest resolution RT-nucleic acid structure to date.

The HIV-1 RT-aptamer complex is catalytically active and can serve as a platform for studying fundamental RT mechanisms and for development of anti-HIV inhibitors through fragment screening and other approaches. In addition to the binary structure (a "P site" complex), we have also determined structures of (1) HIV-1 RT/aptamer as a catalytic ternary complex with incoming AZT-triphosphate, (2) an "N site" structure following the incorporation of AZT at the primer terminus prior to translocation, and (3) a complex with the pyrophosphate-mimic drug foscarnet (phosphonoformate).

We are grateful for support from NIH grants P50 GM103368 (to EA, and a Collaborative Development Grant to JD) and NIH MERIT Award grant R37 AI027690 (to EA).

BACK

Visualization and Animation of the HIV Life Cycle

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In collaboration with the HIVE and CHEETAH Centers, we have created visual materials to simulate a view of the HIV life cycle. We have taken two complementary approaches, creating illustrations of HIV in its cellular environment at key steps in the life cycle, and capturing the dynamics of viral attachment, entry and exit in detailed animations. These illustrations and animations are based on structural and ultrastructural information from diverse sources, providing an integrated view of the processes. Our goal is to create visual materials that may be used as thinking tools to assess the current state of knowledge, and for use in education and public outreach. These materials are freely available at: http://hive.scripps.edu/resources.html and http://scienceofhiv.org

Recruitment of HIV genomic RNA

Alice Telesnitsky

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HIV-1 RNA's 5' leader orchestrates several replication functions, including specific RNA packaging. In the years since "wreck and check" approaches first implicated the HIV-1 5' leader in packaging, many studies addressing packaging determinants and models for this RNA's secondary structure have been reported, without strong consensus. The three-dimensional structure of the HIV-1 RNA packaging signal, solved by Summers' group with input from CRNA investigators Case, Johnson, Salemi, and Telesnitsky, now provides new insight into how nascent HIV-1 RNAs are recruited for packaging.

This new structure makes functional predictions that are testable using targeted viral mutants. Specifically, earlier suggestions that HIV-I displays *cis*-preference in RNA packaging can be explained by unintended changes in RNA folding that resulted from alterations to the *gag* start codon. Spliced RNAs are not packaged even though they possess dimer initiation sequences because they lack the RNA elements that present these motifs to dimerization partners. RNAs in the packaging-competent form are not spliced because they exist in a radically different structure than previously inferred from chemical probing data. The NMR structure is further validated by almost complete loss of RNA packaging upon mutagenesis of unpaired 5' leader G residues implicated as NC binding sites in recent CLIP studies by CRNA investigators Kutluay and Bieniasz.

One complication in defining its packaging elements has been HV-1's packaging promiscuity. Unlike retroviruses like MLV, for which possession of packaging sequences confers a ~1000X packaging advantage, HIV-1 "Y-" RNAs are packaged 20-50% as well as intact gRNAs. However, selectivity for HIV 5' leader sequences is confirmed in competition experiments, in which Y+ RNAs are packaged >20X better than Y- RNAs.

Although HIV-1 Y- RNAs are poorly packaged in the presence of authentic 5' leader RNAs, they are packaged significantly better than many other RNAs. Work defining regions outside Y that contribute to HIV-1 packaging suggest an extended RRE fragment is sufficient to account for the non-Y packaging determinants in HIV-1 genomes. The question of whether or not RRE functions in ways other than facilitating nuclear export appears to be answered in the negative by observations that replacing RRE by an MPMV CTE yields RNAs that are packaged well. However, cellular fractionation reveals that some minimal genomes with RREs, which are packaged poorly, are largely cytoplasmic. In summary, new structural work reveals how the dimeric HIV-1 genome interacts with Gag to become specifically encapsidated. Cell biologic aspects of the process remain less well defined.

Electron Cryo-Microscopy of HIV RNA Fragments

Wah Chiu[1], Kaiming Zhang[1], Zhaoming Su[1], Rossi Irobalieva[1], Jan Marchant[2], Sarah Keane[2], Xiao Heng[2,3], David Case[4], Steven J. Ludtke[1], Michael F. Schmid[1], and Michael F. Summers[2]

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Electron Cryo-microscopy (cryoEM) has emerged as an atomic resolution structure tool for large molecular machines such as viruses, chaperonins, enzymes and membrane proteins. For such studies to be successful, particles must produce sufficient contrast, which to date have required molecular masses of at least 200 kDa. We are exploring the possibility of using cryoEM as a tool to determine the structures of HIV RNA fragments including DIS RNA dimer (~30kDa) and RRE (~77kDa). DIS is the crucial segment for the initiation of HIV-1 virion packaging, while the RRE is responsible for exportation of unspliced and alternatively spliced HIV-1 mRNAs out of the nucleus. Challenges of working with these HIV RNA samples are their potential structural flexibility and low contrast due to small size. We have employed a number of the latest instruments and software advances in cryoEM to pursue our studies. Our image processing analysis clearly demonstrated the presence of multiple conformers of these RNAs. Through extensive classification processes, a subset of image data has been obtained for both of these RNAs to produce an 18 Å map for the DIS RNA dimer and 22 Å for the RRE. In the case of DIS RNA, we have found a dimeric and pseudo 2-fold feature in one subset of particles. In the case of RRE, the primary population of the RNA images appears different than the previous model. These results have confirmed the dynamic behavior of small RNAs in solution, as well as posed challenges and opportunities in RNA structural biology.

A Dual Structural Mimicry Enables Expression of the HIV-1 Genome

Carolina Salguero, Vincent V. Pham, Shamsun N. Khan, Nicolas Humbert, Hugues de Rocquigny and Victoria M. D'Souza

Complete synthesis of the HIV genome requires release of a stalled transcriptional elongation complex by the positive elongation factor b (pTEFb). This process necessitates the interaction of the viral Tat protein with the cellular 7SK small nuclear RNA (7SK snRNA), which keeps pTEFb sequestered via the HEXIM protein. I'll show that the 7SK stem-loop 1 is peppered with arginine sandwich motifs that are uniquely engaged by Tat's RNA binding domain (RBD). Arginine residues within the RBD are effectively positioned to intercalate into these multiple motifs, allowing Tat to intimately dock deep into the major groove of 7SK with high specificity and affinity. I'll further define the role of the critical residue R52 as the molecular key that remodels one of the motifs from a locked pseudo configuration to a classical sandwich. This conformational switch may be the molecular mechanism by which Tat and other cellular factors displace HEXIM for pTEFb release.

BACK

The HARC Center: Progress and Overview

Yifan Cheng[1,2,3], Charles Craik[2,4], Jennifer Doudna[2,3,5], Alan Frankel[1,2], John Gross[2,4], Matt Jacobson[2,4], Nevan Krogan[2,6], Matija Peterlin[7], and Robert Stroud[1,2]

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The mission of the HARC Center has been to obtain a structural picture of HIV-host complexes involved in regulatory and accessory functions, to increase mechanistic understanding of the virus life cycle and identify new targets and protein interfaces for intervention. To accomplish this, we have created a diverse team of investigators and collaborators that span the breadth of expertise needed to achieve a “systems-to-structure” pipeline to elucidate an understanding of HIV-host biology and structure. At the heart of the HARC Center are technological approaches to help identify and characterize HIV-human protein complexes, including cryo-EM, mass spectrometry, computational techniques, CRISPR-based genetic strategies and Fab-based biochemistry. We have been utilizing these approaches to study several HIV proteins, including Tat, Rev, Vif and Nef. These studies are providing a much deeper understanding of how the virus subverts the host machinery to control transcription, RNA trafficking, protein degradation, and other essential cellular processes. Updates of several of these ongoing works will be discussed.

HIV-1 Nef Hijacks Clathrin Coats by Stabilizing AP-1 Polygons

Qing-Tao Shen[1], Xuefeng Ren[1], Rui Zhang[1,2], Yifan Cheng[3], Il-Hyung Lee[1] and James H. Hurley[1,2]

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HIV Nef downregulates cell surface proteins by hijacking the AP-1 and -2 clathrin adaptors. We show that HIV-1 Nef cooperates with the GTPase Arf1 to induce the oligomerization and activation of AP-1. We report the cryoEM structure of an HIV-1 Nef and Arf1-bound AP-1 assembly at 8 Å resolution. This cryoEM structure, when combined with the previously determined crystal structure of an AP-1:Arf1 dimer, led us to predict the formation a 40 nm-diameter AP-1 hexagons whose inner face contains binding sites for membrane lipids and proteins, whilst the outer face provides a platform for clathrin assembly. This prediction was validated by mutational analysis of Arf1- and Nef-promoted clathrin cage assembly in vitro. Arf1 and HIV-1 Nef thus play interconnected roles in allosteric activation, cargo recruitment, and coat assembly, revealing an unexpectedly intricate organization of the inner AP-1 layer of the clathrin coat.

BACK

Resolution and Inhibition of Vif-APOBEC3 Interactions

Jennifer Binning[1], Natalia Sevillano[1], Hai Ta[1], Florencia La Greca[1], Judd Hultquist[2,3], Amber Smith[4,5], Nathalie Caretta Cartozo[1], Nevan Krogan[2,3], Yifan Cheng[4,5], Charles Craik[1], and John D. Gross[1]

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The APOBEC3 (A3) family of restriction factors protects hosts from retroviruses and retroelements. They are polynucleotide cytosine deaminases that restrict by mutating viral cDNA. The lentiviral Vif protein promotes spread of virus in the host by targeting A3 family members for degradation by the 26S proteasome. To do so, Vif must hijack an E3 ligase from the Cullin-RING ligase (CRL) superfamily. In primates, the function of the Vif-E3 requires a non-canonical CRL subunit, core-binding factor beta, whereas this requirement is absent or different in non-primate species. Structural studies of full-length human A3 restriction factors in complex with the Vif-E3 are important because they could shed light on a molecular arms race that gave rise to the AIDS pandemic. Agents that inhibit Vif-A3 interactions would restore the restriction potential of A3 family members and be useful as anti-HIV therapeutics. Here we report three key results. First, we have reconstituted and characterized human A3 substrates in complex with the HIV-1 Vif holoenzyme. Second, using phage display, we have discovered high-affinity fragment antigen-binding (Fab) fragments for the HIV-1 Vif complex to facilitate structural studies with A3 substrates. Third, we demonstrate that one Fab can inhibit Vif activity in vitro and in cells, blocking neutralization of A3F and A3C but not A3G. Our findings document a useful strategy for resolving and inhibiting Vif complexes, and for the first time experimentally establish the feasibility of generating inhibitors that directly target a specific interface of HIV Vif.

HIV-1 RNA Genome: the Journey and the Destination

Jianbo Chen[1], David Grünwald[2], Luca Sardo[1], Olga Nikolaitchik[1], Sheikh Abdul Rahman[1], Ryan C. Burdick[3], Andrea Galli[1], Sergey Plisov[1], Sheldon Tai[1], ,Edward Liang[1], De Chen[4], Stephen Lockett[4], Daniel R. Larson[5], Vinay K. Pathak[3], and Wei-Shau Hu[1]

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HIV-1 full-length RNA plays a central role in viral replication, serving as the template for Gag/Gag-Pol translation and as the genome for the progeny virus. To carry out these functions, HIV-1 RNA needs to be transported to appropriate subcellular compartments and interacts with other viral molecules. We sought to determine the mechanism used to transport HIV-1 RNA in the cytoplasm, the dynamics of RNA near the plasma membrane, and the interactions of RNA genomes with other viral molecules. Our results from live-cell imaging studies will be discussed.

BACK

The Onset of Viral Assembly and the Interaction of Endogenous ESCRT Proteins with HIV Assembly Sites Investigated Using Advanced Fluorescence Microscopy

Don C. Lamb[1], Jelle Hendrix[2], Jens Prescher[1], Viola Baumgärtel[1], Hans-Georg Kräusslich[3], and Barbara Müller[3]

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Assembly of the Gag polyprotein into new viral particles in infected cells is a crucial step in the retroviral replication cycle. It has been speculated that Gag forms oligomers in the cytosol before assembling at the plasma membrane. To investigate the cytosolic HIV-1 Gag fraction in real time in live cells, we used advanced fluctuation imaging methods. We could show that Gag diffuses as a monomer on the subsecond timescale with severely reduced mobility. Reduction of mobility is associated with basic residues in its nucleocapsid (NC) domain. Another diffusive Gag species was observed on the seconds-timescale that oligomerized in a concentration-dependent manner. Our results reveal potential nucleation steps of cytosolic Gag fractions prior to membrane-assisted Gag assembly.

Another crucial step in the retroviral replication cycle is budding of the nascent virus particle. During the budding process, the cellular endosomal sorting complex required for transport (ESCRT) machinery is hijacked by HIV-1. We used super-resolution fluorescence microscopy to investigate the interactions of ESCRT proteins with HIV-1 assembly sites and measured the size and structure of the ESCRT components Tsg101, ALIX, CHMP4B and CHMP2A. To avoid the deleterious effects of using fusion proteins attached to ESCRT components, we performed measurements on the endogenous protein. In the case of CHMP4B, constructs modified with the small HA tag were used. All colocalizing ESCRT clusters exhibited closed, circular structures with an average size (full-width at half-maximum) between 45 and 60nm or a diameter (determined using a Ripley's L-function analysis) of roughly 60 to 100nm. The size distributions for colocalizing clusters were narrower than for non-colocalizing clusters and significantly smaller than the HIV-1 bud. Hence, our results support a membrane scission process driven by ESCRT protein assemblies inside a confined structure, such as the bud neck, rather than by large lattices around the neck or in the bud lumen.

Computational Modeling of the Immature Retroviral Lattice

Boon Chong Goh[1], Juan R. Perilla[1], Matthew R. England[2], Katrina J. Heyrana[2], Rebecca C. Craven[2] and Klaus Schulten[1]

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Obtaining an atomic structure of the immature Gag lattice has been elusive for many years. Recent advances in cryo-electron microscopy have yielded high-resolution density maps and therefore enabled accurate computational modelings and simulations. Computational modeling offers a unique opportunity to investigate the physical and chemical processes that occur during the replication cycle of a virus. In this poster, we present the first atomic model of an immature Gag lattice (1), using Rous Sarcoma Virus as the model system. This model was obtained using microsecond-long molecular dynamics simulations and tested by mutagenesis experiments in vitro. In light of our results, we discuss the recently published electron micrograph of the immature capsid of HIV.

1. BC Goh, JR Perilla, MR England, KJ Heyrana, RC Craven, and K Schulten, Atomic Modeling of an Immature Retroviral Lattice using Molecular Dynamics and Mutagenesis, *Structure*, (accepted)

BACK

Crystal Structures of Native and Mutant HIV-1 Capsid Proteins Reveal Molecular Details of Interactions with Ligands and Structural Basis of Capsid Stability

Anna T. Gres[1,2], Karen A. Kirby[1,3], Dandan Liu[1,3], Thomas G. Laughlin[1,3], Vineet N. KewalRamani[4], John J. Tanner[2,5], Owen Pornillos[6], and Stefan G. Sarafianos[1,3,5]

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The HIV-1 genome is enclosed by a capsid formed by ~1,600 copies of a single capsid protein (CA) arranged in ~250 hexamers and 12 pentamers. During the viral life cycle, CA interacts with several host factors including CPSF6 that affects capsid stability and nuclear import pathway. PF74 is a CA-targeting potent antiviral that at low concentrations affects CPSF6 binding, nuclear entry, and at higher concentrations also blocks uncoating, assembly and reverse transcription. In addition, the CA E45A mutation stabilizes the capsid, delays uncoating and impairs infectivity.

The structures of CA and relevant CA assemblies have been studied extensively over the past 20 years. The hexagonal lattice of the capsid has been shown to comprise multiple interfaces at the 6-, 3-, and 2-fold symmetry axes. CA-CA interactions at these interfaces affect multiple stages of the viral life cycle; hence, the molecular details of these interactions are critical.

We recently solved structures of native CA crystallized in space group P6 with a single CA molecule per asymmetric unit. These structures provided molecular details on the inter-hexamer interactions in the context of a native CA protein. This information improves our understanding of how CA builds a hexagonal lattice, the foundation of the mature capsid. They also reveal an unexpected adaptable hydration layer that modulates interactions among CA molecules. CA structures in complex with CPSF6 or PF74, in addition to binding sites, reveal how ligand binding changes inter-hexamer interactions. Specifically, helices H10 at remote 3-fold interface converge closer, replacing water molecules from the interface, thus affecting capsid stability. We also solved the structure of CA E45A, which shows local changes at the site of mutation and increased number of interactions at the 2-fold and 3-fold interfaces. Surprisingly, CA E45A revealed an additional 3-fold *inter-hexamer* interface formed by R82 of the *N-terminus domain* of the protein that may explain hyperstabilization of the capsid bearing this mutation. Collectively, our structures reveal an inherent structural plasticity of the capsid, which is required to fulfill its diverse roles.

Structural Studies of APOBECs

Celia Schiffer

UMass Medical School

Our project team has determined the experimental structures of four APOBEC3 domains whose atomic structures have been determined. We have also modeled the remaining domains. These structures provide us insights into how these domains recognize specific substrates, bind HIV-1 Vif and oligomerize, and detailed through structural comparisons we begin to gain insights into the molecular mechanisms by which these enzymes distinguish their functions and can potentially be specifically targeted by therapeutics.

Structure of the Vif-Binding Domain of the Antiviral Enzyme APOBEC3G

Takahide Kouno[1,2], Elizabeth M. Luengas[3], Megumi Shigematsu[1,4], Shivender M.D. Shandilya[2], JingYing Zhang[1,4], Luan Chen[1,4], Mayuko Hara[1,4], Celia A. Schiffer[2], Reuben S. Harris[3] and Hiroshi Matsuo[1]

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The human APOBEC3G (A3G) DNA cytosine deaminase restricts and hypermutates DNA-based parasites including HIV-1. The viral infectivity factor (Vif) prevents restriction by triggering A3G degradation. While the structure of the A3G catalytic domain is known, the structure of the N-terminal Vif-binding domain has proven more elusive. Here, evolution- and structure-guided mutagenesis was used to solubilize the Vif-binding domain of A3G permitting structural determination by NMR spectroscopy. A smaller zinc-coordinating pocket and altered helical packing distinguish it from catalytic domain structures, and help explain the reported inactivity of this domain. This soluble A3G N-terminal domain is bound by Vif, which enabled mutagenesis and biochemical experiments to identify a unique Vif-interacting surface formed by α1-β1, β2-α2, and β4-α4 loops. This structure sheds new light on the Vif-A3G interaction and provides critical information for future drug development.

Chemical Probes of APOBEC3G Deamination

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APOBEC3 (A3) enzymes are single-stranded DNA cytosine-to-uracil deaminases that function as part of the innate immune response to pathogens. APOBEC3G (A3G) restricts HIV-1 infection in the absence of Viral Infectivity Factor (Vif), which targets A3G for proteasomal degradation. However, even in the presence of Vif, A3G promotes sub-lethal mutations that enable the virus to evolve and evade drug therapies (1). Given the foundational role of A3G in host-pathogen interactions, we have begun developing chemical probes of the A3 family of enzymes.

High-throughput screening and subsequent chemical optimization and biochemical assays have identified two classes of covalent inhibitors of A3G, which are represented by small molecules MN30 and MN256 (2,3) These chemical probes have enabled the identification of cysteine-321 on A3G as a susceptible binding site of electrophilic compounds that result in inhibition of enzymatic activity when targeted. Additionally, these early probes have served as launch points for the development of second-generation small molecule inhibitors with improved physiochemical properties. The discovery of MN30 and MN256 as well as progress towards the development of additional analogues will be presented.

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BACK

The Interdependency of Drug Resistance Evolution and Drug Design: HIV-1 Protease, a Case Study: Project Overview

R. Swanstrom[2], W. Sherman[3], A. Ali[1], K. Zeldovich[1], N. Kurt-Yilmaz[1], S Lee[2], R. Ishima[4], J. Luban[1], D. Bolon[1], and C. Schiffer[1]

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In this project we are elucidating the underlying interdependencies under which drug resistance is selected using HIV-1 protease as a model system. Drug resistance occurs when the balance favors viral replication between proteolytic activity within the virus and enzyme inhibition. We combine analysis of sequence diversity, structure, dynamics and energetics to elucidate the underlying pathways and additional macromolecular pressures that contribute to drug resistance.

BACK

A Direct Interaction with RNA Dramatically Enhances the Catalytic Activity of the HIV-1 Protease In Vitro

R. Swanstrom[2], W. Sherman[3], A. Ali[1], K. Zeldovich[1], N. Kurt-Yilmaz[1], S Lee[2], R. Ishima[4], J. Luban[1], D. Bolon[1], and C. Schiffer[1]

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The HIV-1 protease can interact with RNA to accelerate the rate of cleave between 10 and 100 fold. There is an electrostatic interaction but with some element of sequence/structural specificity. Gel-shift assays demonstrated the HIV-1 PR is capable of interacting with nucleic acids, suggesting RNA accelerates processing reactions by interacting with the PR rather than the substrate. All HIV-1 PRs examined have this ability. This interaction could contribute to regulation of timing of proteolysis during the virion assembly process.

Free Energy Perturbation Predicts the Energetics of Resistance

R. Swanstrom[2], W. Sherman[3], A. Ali[1], K. Zeldovich[1], N. Kurt-Yilmaz[1], S Lee[2], R. Ishima[4], J. Luban[1], D. Bolon[1], and C. Schiffer[1]

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The ability to predict protein-ligand binding free energies is central to structure-based drug design. To understand drug resistance, we not only have to predict binding energies to the wild-type protein but also potential resistance mutations. We have made recent advances in the free energy perturbation (FEP) method that have resulted in reliable predictions of protein-ligand binding energies for congeneric molecules. Here, we expand the application of this method to encompass protein mutations and show that we can achieve a high level of accuracy. We present applications of protein FEP in the context of improving binding energies and predicting the energetic effects of mutations.

Mutations Outside the Active Site and Co-Evolution of Substrates Confer Drug Resistance Through Alterations in the Dynamic Network and Structural Ensemble of HIV-1 Protease

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With few exceptions, the role of mutations outside the active site and within the substrates in conferring resistance remains largely elusive. Through a series of DRV-protease complex crystal structures, inhibition assays, and molecular dynamics simulations, we find that single and double site mutations outside the active site often associated with DRV resistance alter the structure and dynamic ensemble of HIV-1 protease active site. We also find that analyses of the protease-substrate interactions reveal that compensatory coevolved mutations in the substrate also establish other interactions that are not restricted to the site of mutation. Mutation of a substrate residue induces a conformational change in the protease. Molecular dynamics simulations suggest that restoration of active site dynamics is an additional constraint in the selection of coevolved mutations. Thus, protease evolution permits structural, and dynamic changes via molecular mechanisms that involve distal effects contributing to drug resistance.

BACK

The Structure of a CXCR4:Chemokine Complex with Insights into Chemokine Receptor Recognition by gp120

Ling Qin, Irina Kufareva, Chong Wang, Huixian Wu, Bryan Stephens, Lauren Holden, Beili Wu, Gus Fenalti, Vadim Cherezov, Ray Stevens, Ruben Abagyan, and Tracy Handel

Chemokine receptors are G Protein-Coupled Receptors (GPCRs) best known for their role in controlling cell migration in the context of immune system function. However, inappropriate regulation of chemokine-mediated processes contributes to the pathology of many diseases, the most validated of which is HIV where CCR5 and CXCR4, serve as co-receptors for viral entry into cells. Chemokines/receptors are also associated with a multitude of inflammatory diseases such as asthma, rheumatoid arthritis, and multiple sclerosis. For these reasons, they have become widespread targets in the pharmaceutical industry where high-resolution structures would be extremely valuable for drug design. In the last several years, numerous structures of G Protein-Coupled Receptors (GPCRs) have finally succumbed to crystallization including two chemokine receptors in complex with small molecule antagonists. However, the natural ligands of chemokine receptors are small proteins, and protein:protein complexes involving membrane proteins tend to be particularly challenging structural targets. In this presentation I will describe our strategy for determining the structure of the chemokine receptor, CXCR4, in complex with a chemokine ligand and what we have learned from the structure. In particular, I will show the analogies we envision between CXCR4:chemokine and CXCR4:gp120 interactions.

Structure of the Rous Sarcoma Virus Intasome

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Integration of the reverse-transcribed viral DNA into the host genome is an essential step in the lifecycle of retroviruses, which establishes permanent viral infection. Retrovirus integrase (IN) catalyzes insertions of both ends of the linear viral DNA into a host chromosome. IN from HIV-1 and closely related retroviruses share the canonical three-domain organization, consisting of a catalytic core domain flanked by N- and C-terminal domains essential for the concerted integration reaction. Although structures of the tetrameric IN-DNA complexes have been reported for IN from prototype foamy virus (PFV) featuring an additional DNA-binding domain and longer interdomain linkers, the architecture of a three-domain IN bound to DNA remained unknown. We have determined crystal structure of a three-domain IN from Rous sarcoma virus (RSV) in complex with viral and target DNAs. The structure shows an octameric assembly of IN, which is held together through extensive network of protein-DNA and protein-protein contacts distinct from those observed for PFV IN. Our work highlights diversity of retrovirus intasome assembly and provides insights into the mechanisms of integration by HIV-1 and related retroviruses.

BACK

Program Summary and Advancements in Env gp120 Inhibitor Designs and Structural Mechanisms

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Our Program is focused on investigating strategies to inhibit HIV-1 gp120 in the metastable virus envelope glycoprotein (Env) protein trimer, identifying and developing gp120 inhibitory compositions, and using inhibitors to define fundamental mechanisms and targetable vulnerabilities in Env. Several new types of low-molecular-weight gp120 inhibitors have been advanced. These include: [1] small-molecule CD4 mimetics that bind the highly conserved CD4 Phe 43 binding pocket with improved affinity; [2] activation blockers such as BMS-806 and 18A that target a neighboring conserved site; and [3] proteolytically stable macrocyclic peptide triazoles that inactivate HIV-1 and target a conserved 2-cavity site containing components of both the CD4 mimetic and activation blocker sites. Determination of structural modes of binding with monomeric gp120 and trimeric forms of Env has guided definition of structural mechanism, synthetic design and acquisition of improved inhibitor potency. Our program advances methodologies to evaluate both the biophysical and virological effects of inhibitors on isolated Env protein conformation and Env on viruses and cells. Several translational directions have been identified, including [1] small-molecule CD4 mimetics that sensitize Env for interactions with neutralizing and ADCC-mediating antibodies, and thus have potential use in enhancing vaccine efficacy, and [2] macrocyclic peptide triazoles for which metabolic stability opens up opportunities for small animal anti-HIV-1 therapeutics testing.

BACK

Antibodies Elicited by Multiple Envelope Glycoprotein Immunogens in Primates Broadly Neutralize Human Immunodeficiency Viruses (HIV-1) Sensitized by CD4-Mimetic Compounds

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Preventing sexual transmission of human immunodeficiency virus (HIV-1) is essential for altering the course of the global pandemic of acquired immunodeficiency syndrome (AIDS). Antibodies that bind the HIV-1 envelope glycoproteins (Env) spike neutralize virus infectivity and have been demonstrated to protect passively in monkey models of HIV-1 infection. However, current vaccine candidates do not elicit antibodies that bind to conserved elements of the Env spike and neutralize multiple primary HIV-1 strains. Structural features of the unliganded HIV-1 Env trimer decrease the elicitation and impact of antibodies; thus, even the best current Env-based vaccines elicit antibodies that neutralize only the more neutralization-sensitive subset of primary HIV-1 variants. We have identified small molecules CD4 mimetics that bind to a highly conserved pocket on the HIV-1 gp120 Env and dramatically sensitize HIV-1 to vaccine-elicited antibodies. We have investigated the range of antibodies whose neutralizing potency is enhanced by these small molecules.

BACK

Conformational Dynamics of Single HIV-1 Env Trimers on Native Virions

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The HIV-1 envelope (Env) mediates viral entry into host cells. To enable the direct imaging of conformational dynamics within Env, we introduced fluorophores into variable regions of the glycoprotein gp120 subunit and measured single-molecule fluorescence resonance energy transfer within the context of native trimers on the surface of HIV-1 virions. Our observations revealed the unliganded HIV-1 Env to be intrinsically dynamic, transitioning between three distinct prefusion conformations, whose relative occupancies were remodeled by receptor CD4 and antibody binding. The distinct properties of neutralization-sensitive and neutralization-resistant HIV-1 isolates support a dynamics-based mechanism of immune evasion. Broadly neutralizing antibodies VRC01, PG16, PGT128, PGT122, PGT145, and 2G12, and the entry inhibitor, BMS-626529, stabilize the ground state of Env. These data indicate that most broadly neutralizing recognize the ground state and suggest that ground state stabilization represents an effective strategy to antagonize viral fusion machines.

BACK

T1. Retrovirus Dissemination Depends on CD169/Siglec-1 Mediated *Trans*-Infection of Permissive Lymphocytes

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Dendritic cells can capture and transfer retroviruses *in vitro* across synaptic cell-cell contacts to uninfected cells, a process called *trans*-infection. Whether *trans*-infection contributes to retroviral spread *in vivo* remains unknown. Here we visualize the dissemination of retroviruses in secondary lymphoid tissues of living mice. We demonstrate that murine leukemia virus (MLV) and human immunodeficiency virus (HIV) are first captured by sinus-lining macrophages. Virus capture is mediated by CD169/Siglec-1 that recognizes viral gangliosides. MLV-laden macrophages then form long-lived synaptic contacts to *trans*-infect B-1 cells. Infected B-1 cells subsequently migrate to the inter-follicular space to spread the infection through virological synapses. Efficient infection of mice requires CD169, suggesting that retroviruses utilize a combination of fluid-based movement followed by CD169-dependent *trans*-infection to spread *in vivo*.

T2. Characterization of Novel Mutations in the HIV-1 Env Glycoprotein that Globally Rescue Defects in Virus Replication

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Infectious HIV-1 particle production is driven by the expression of the Gag polyprotein precursor that acts to recruit host cell factors to assist in viral budding. The p6 domain of HIV-1 Gag contains a YPX_nL motif, a “late” domain, which promotes the release of virions from infected cells through a direct interaction with the ESCRT-associated protein Alix. We previously demonstrated a functional role of Gag p6-Alix binding in HIV-1 particle production and replication by introducing mutations into p6 and characterizing subsequent alterations in virus replication kinetics. The most striking defect observed was a severe delay in virus replication induced by these mutations. To ascertain the nature of this replication defect, we passaged these replication-defective Alix-binding site mutant viruses in culture and selected for viral isolates with near-wild-type replication kinetics. Sequencing of the viral revertants revealed novel mutations in Env located near the N-terminus of gp120, in the gp41 heptad repeat region, and in the cytoplasmic tail of gp41. These mutations confer full rescue of the original replication-defective Gag mutants in Jurkat T-cells; however, this rescue does not correlate with an enhancement of cell free, single-cycle infectivity. To explain this phenomenon, we performed biochemical assays to characterize the mechanism of rescue of the Env compensatory mutations. We demonstrate that these Env mutations do not enhance Env expression, Env incorporation, or virus release efficiency. Some of the Env mutants exhibit a decrease in fusogenicity while all but one exhibit an impairment in single-cycle infectivity as compared to wild type in both TZM-bl and Jurkat 1G5 target cells. Interestingly, at least one of the mutations is capable of rescuing a non-budding related replication defect, suggesting that these compensatory mutations assist in a global rescue of viral fitness. Vertical scanning mutagenesis of these Env residues implies a continued discordance between replication fitness and single-cycle infectivity. Interestingly, replication kinetics of the original replication-defective Gag mutants, Env compensatory mutations, and p6/Env double mutants in another T-cell line, CEM 12D7, correlates with the defects observed in single-cycle infectivity in Jurkat cells. Therefore, while these Env mutations have no significant effect on cell-free transmission, we instead propose that they rescue the replication-defective Gag p6 mutants by enhancing cell-to-cell viral transmission, specifically in Jurkat T-cells. Cell-to-cell HIV-1 transmission occurs more efficiently and rapidly than infection by cell-free viruses, supporting the relevance of this mode of viral dissemination. We are currently further investigating the role of Env in cell-to-cell virus transfer.

BACK

T3. Incorporating Cheminformatic Predictive Tools into Structure-Based Discovery of CCR5 Antagonists

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The discovery of Highly Active Antiretroviral Therapy (HAART) has made it possible for HIV infected patients to live long and relatively healthy lives and has given researchers hope for fighting the disease. However, complete eradication of HIV/AIDS remains a scientific challenge. Although there are multiple approved antiretroviral drugs that can halt replication of the virus, these drugs are riddled with drug resistance and antiretroviral (ARV) toxicity. Human Chemokine Receptor 5 (CCR5) is a member of a large family of 20 chemokine receptor subtypes. A major role of CCR5 is as a co-receptor for trafficking HIV virion into cells. It is a viable drug target for inhibiting HIV replication as is evident by Maraviroc, a CCR5 antagonists currently on the market for treating HIV. In addition, the identification of a mutant allele of the CCR5 gene (CCR5Δ32) that cripples the ability of CCR5 to act as a co-receptor indicates the significance of the role CCR5 plays in the HIV lifecycle. Herein, we conducted extensive cheminformatic modeling and molecular docking studies to thoroughly evaluate the binding profile of CCR5 antagonists. Categorical and continuous cheminformatic models were built using advanced machine learning algorithms such as random forest (RF), support vector machines (SVM), and Genetic algorithm *k*-Nearest Neighbor (GA-*k*NN). Only robust models with highly predictive ability were chosen for virtual screening of TimTec and ChemBridge chemical libraries to identify novel CCR5 inhibitors. That serves as an important high-throughput filter prior to the molecular docking refinement. The crystal structure of CCR5 in complex with Maraviroc, was used for rigid docking of 35 QSAR hits with GOLD software. Our results identified 3 potential CCR5 inhibitors with similar binding profiles as that of Maravoric indicated by the comparable Gold Chemscores. Molecular docking is used to complement QSAR modeling and increases the reliability of the QSAR modeling results.

T4. Structural Targeting of Potentially Protective HIV-1 gp120 Epitopes in the C1-C2 Region

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Although existing evidence confirms a key role of non-neutralizing antibodies (nnAbs) targeting the C1-C2 region of gp120 in antibody-dependent cell-mediated cytotoxicity (ADCC) responses to natural infection, protection in NHP models and the RV144 vaccine trial in humans, the exclusive role of nnAbs specific for this epitope target in protection has never been explored directly in vaccine settings mostly due to the lack of an appropriate immunogen. Here we describe a structure based design of a novel immunogen, ID, aiming to solely stimulate humoral responses involving FcR-effector mechanisms directed at the C1-C2 epitope region and without complication of neutralizing activities. ID incorporates the C1-C2 epitopes within a minimal structural unit of gp120 and consists of the inner domain of the gp120 core stabilized in CD4-bound conformation expressed independently of the outer domain. The ID design was guided by our recent atomic level description of the A32 epitope sub-region gained from crystal structures of several A32-like antibodies in complexes with the CD4-triggered gp120 cores. ID stably expresses the C1-C2 epitopes involved in potent FcR-effector function to HIV-1 as indicated by structural, antigenicity and functional testing. When injected into rabbits, ID elicits the A32-like antibody response with ADCC activities. ID is a novel immunogen candidate capable of selective induction of humoral responses involving FcR-effector function permitting the address, for the first time, of the exclusive role of nnAbs in protection from HIV-1 in vaccine settings.

T5. Microscopic Detection and Localization of HIV gp120 Epitope Exposures on Cell-Bound Virions

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The pathways of protein domain exposure on the HIV surface delimit relationships between infection and humoral immunity and provide an important framework for developing approaches to treat or prevent HIV infection. The gp120 and gp41 components of HIV envelope trimers have been intensely studied in the context of free virions and it is understood how these proteins must experience multiple structural rearrangements during the course of host cell receptor (CD4 and coreceptor) engagement. However, the impact of such changes on the structural features of an intact virion bound to a host cell remains an ongoing, critical question. Previous predictions held that conserved envelope domains operating within cell contact zones encounter a variety of spatial constraints that occlude their exposure and/or immunoreactivity. However, certain lines of evidence are discordant with these predictions. Although transition state gp120 domains absent on free virions but induced by CD4 binding (CD4i) are predicted to be profoundly occluded on cell-bound HIV, cognate anti-CD4i monoclonal antibodies reproducibly mediate antibody dependent cell cytotoxic activity (ADCC) against target cells presenting attached virions. Prompted by such discrepancies, we applied confocal and three-dimensional superresolution microscopic techniques to interrogate virions bound to entry-competent target cells. Surprisingly, these analyses showed that CD4i epitopes on gp120 are visibly exposed and reactive with whole cognate antibodies on bound virion surfaces for a period of hours. These exposure patterns resemble what is observed for constitutively expressed neutralizing epitopes on cell-bound virions. Further, three-dimensional direct stochastic optical reconstruction microscopy (dSTORM) showed that CD4i epitopes were unexpectedly exposed distal to the HIV- cell contact interface in a manner readily accessible to circulating antibodies. Such distal exposures of certain CD4i epitopes was abrogated on mutant virions with aberrant matrix/capsid structures. Collectively these observations suggest that previously unsuspected structural dynamics emerge on HIV during host cell attachment. Such processes may provide new windows of vulnerability to antiviral countermeasures against freshly targeted host cells.

BACK

T6. Understanding Glycan Type Specificity in Highly Glycosylated Proteins

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Despite the fact that the vast majority of glycans in most known glycoproteins are of complex type, some glycoproteins have a clear tendency to contain high-mannose and hybrid structures as well. For example, HIV envelope glycoprotein gp120 usually has about 24 glycans, and almost half of them are either high-mannose or hybrid. Statistical analysis of gp120 suggests that: 1) glycosylation sites placed closer to the C-terminus of the protein tend to carry high-mannose/hybrid glycans; 2) glycosylation sites placed in flexible loops tend to carry complex glycans; and 3) glycosylation sites placed in regions of secondary structure tend to carry high-mannose/hybrid glycans. Analysis of glycosylation sites placed in other fucose-containing glycoproteins' secondary structure was inconclusive, though our results for highly glycosylated proteins were consistent with our hypothesis. In order to develop a mechanistic understanding of the origin of these differences, we developed a stochastic chemical-kinetic model of the N-linked glycosylation pathway. This model provides some understanding of how the distribution of glycan types depends on: 1) concentration of glycans; and 2) parameters that describe certain glycan modifications.

BACK

T7. Optimization of Small-Molecule CD4-Mimetic HIV-1 Entry Inhibitors

Francesca Moraca[1,2,3], Bruno Melillo[3], Cameron F. Abrams[1,2], Wayne Hendrickson[4], Shuaiyi Liang[4], Navid Madani[5], Joseph Sodroski[5,6], and Amos B. Smith, III[3]

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Cellular infection by HIV-1 initiates with the binding of the viral envelope (Env) glycoprotein gp120 and the human cellular receptor CD4. This event results in the exposure of another binding site on gp120, now for co-receptors on the target cell (CCR5 or CXCR4). Co-receptor binding then triggers a conformational cascade that permits partial insertion of Env into the target cell membrane, followed by another refolding event that brings the viral and cell membranes into close proximity to promote fusion. Through a multidisciplinary approach combining computer-aided drug design, organic synthesis and thermodynamic, biochemical and virological studies, we have designed a class of small molecules that bind gp120 similarly to CD4 (Small-Molecule CD4 Mimics, SMCM) and thus disrupt the viral entry process. The derived SMCM scaffolds comprise three pharmacophoric regions: Region I, a dihalogenated phenyl ring; Region II, an oxalamide linker; Region III, variously decorated indane rings.

We here present the synthesis of our two most potent SMCM yet, JP-III-048 and BNM-III-170, in conjunction with a strategy employing docking studies and estimations of free energies of binding to optimize both their affinity to gp120 and their viral neutralization breadth. This strategy forecasts the binding of SMCM to the highly conserved gp120 residues D368 and D474. Specifically, given the strong electrostatic contribution of D368 at the surface of gp120, we considered three protein models to perform our docking studies: *(i)* gp120wt; *(ii)* gp120D368N; and *(iii)* gp120D368 neutralized at physiological pH, with the latter best reproducing the crystallographic binding poses of JP-III-048 and BNM-III-170. In addition, to aid the design of the SMCM congeners, we studied the overlay of CD4 binding residues to the surface of gp120: the comparison of structural and biological data for a range of SMCM designed in our laboratories indicates that high overlap of SMCM Region III with gp120-contacting CD4 segments correlates with increased inhibition of viral entry.

BACK

T8. Investigating Lectin Binding to Envelope Glycoprotein gp120

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Carbohydrate-binding agents (and particularly lectins from various sources) have shown significant promise as prophylactics of HIV infection. While in a general sense it is well-understood that they act by physically interacting with the sugars of the so-called "glycan shield" on the envelope glycoprotein and thus disrupting CD4-mediated cell fusion, a detailed mechanistic understanding of their action is still missing. Some of the unanswered questions include: Are specific glycosylation sites on the envelope preferentially targeted by particular lectins? What is the role of multivalency in the inhibitory mechanism, and is this different for different lectins? Is the primary mechanism of action simply one of steric interference, or does lectin binding perturb the conformational dynamics of the envelope in functionally-relevant ways? We have begun to address these questions from a structural perspective, beginning with a mapping of potential binding sites for various lectins to the envelope glycoprotein gp120 and an assessment of any mutual exclusivity of binding at pairs of sites. We will present the conceptual framework for our approach, as well as preliminary results.

BACK

T9. Macrocyclic HIV-1 Envelope Glycoprotein Antagonists

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We established a facile synthetic methodology to derive macrocyclic HIV-1 antagonists based on a class of structurally minimized linear peptide triazole (PT) inhibitors of the virus Env gp120 glycoprotein. The cyclic PTs (cPTs) retained the ability of the parent PTs to dually inhibit binding of gp120 at both its CD4 and co-receptor binding sites and induce gp120 shedding from the virions, leading to irreversible inactivation. Furthermore, the virolysis effects of PT thiols could be recapitulated with cyclized peptide. These phenotypes argue that cyclization locked the peptide conformation into an active form. The cPTs were able to inhibit HIV-1 pseudovirus cell infection at sub-micromolar concentrations, without host cell toxicity. The 6-residue cPT denoted AAR029b, the most active obtained in this study, inhibited gp120 binding to both sCD4 and the co-receptor surrogate, 17b with IC_{50} ~ 30 nM. AAR029b also inhibited cell infection by both BaL.01 and JR-FL pseudovirus strains with IC_{50} values ~ 200 nM. Importantly, the cyclized peptides were stable to trypsin and chymotrypsin, compared to a substantial susceptibility of corresponding linear PTs to these proteases. Overall, the cPTs are the first macrocyclic inhibitors that target HIV-1 gp120, and therefore represent promising new peptidomimetic leads for developing HIV-1 entry inhibitors, combining the safety and selectivity profiles of peptides with the ease of chemical accessibility and high potency.

T10. Mapping the Conformational Space of Glycoconjugate-Linked Carbohydrates

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It is well understood that carbohydrates and glycoconjugates play crucial roles in biological processes such as coagulation, fertilization, apoptosis and immunity, yet they still remain understudied. Saccharides' conformational flexibility, especially considering their nonlinear backbones, anomeric carbons, and different glycosidic linkage points with various monosaccharide units, increase the difficulty of fully understanding their behaviors in glycan-involved interactions. However, their functional specificity, such as recognition between glycans and other glycans, enzymes or lectins, is highly dependent on their conformational preferences. Hence the accurate prediction of glycan conformations is becoming more and more urgent in carbohydrate chemistry.

In particular, HIV glycoprotein gp120 is densely covered with glycans which account for half of its total mass and several families of broadly neutralizing antibodies (bnAbs) has been discovered showing inhibition for HIV infection in cell culture through binding to different glycan moieties.

Unfortunately, available experimental data is far from enough and interpretations about them have no uniform criteria and bear intrinsic drawbacks. However, using molecular mechanism methods with libraries of peptides rotamers, we are able to generate enough meaningful data and gain insights into structures and functions of proteins and nucleic acids. Hence, to set up libraries for glycans, our approach is to get detailed maps of the free energy landscape for all the biologically important disaccharides and trisaccharides and then extract local minima as initial structures for building oligosaccharide models. Instead of molecular dynamics (MD) simulations which would only visit certain conformations during a typical MD run, we use Monte Carlo (MC) simulation to explore the whole conformational space.

Our preliminary results from disaccharides show clear convergence patterns for glycosidic linkages. Adding one more sugar unit to form linear or three way conjunction trisaccharide only has slight effect on the preferred conformations of their composing linkages.

T11. Interaction Analysis of HIV BG5O5.SOSIP.664 Trimer with Peptide Triazole UM15

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Human Immunodeficiency Virus Type 1 (HIV-1) is a global health problem with over 33 million people infected worldwide and over 2.7 million new infections annually. Host cell infection by HIV-1 is mediated by cell receptor (CD4) interactions with the envelope glycoproteins consisting of a trimeric complex (Env) of gp120 and gp41. Env thus presents itself as an attractive target to attack the virus directly in order to block the cascade of events that lead to host cell infection. Peptide triazoles (PT) are a class of entry inhibitors developed in our lab that put gp120 in an inactive state, suppress the binding of CD4 and co-receptor CCR5, and induce gp120 shedding, leading to complete inhibition of viral entry. However, most of our past biophysical investigations on the interaction mechanism of PT involved monomeric gp120 protein, while the target on the virus is the trimeric spike protein complex. Therefore, here we sought to determine the inhibition mechanism of HIV mediated viral entry using the HIV Env trimeric protein BG505.SOSIP.664. To do this, we utilized a 6 amino acid peptide UM15 that contains the Ile-ferrocenyltriazolePro-Trp (I-X-W) pharmacophore. BG505.SOSIP.664 trimer binding to UM15 was measured using an SPR competition assay. In the presence of increasing concentrations of UM15, a progressive reduction was observed in SOSIP binding to CD4. The interaction between the trimeric Env protein and UM15 was found to be specific, and a calculated IC_{50} value of 280nM was observed. To further elucidate the binding mode of PT onto the trimeric Env protein, *de novo* docking studies were performed in the crystallographically derived BG5O5.SOSIP.664 structure (PDB: 4NCO). These studies showed that UM15 can bind to trimeric Env in a two-cavity region overlapping the CD4 site. In particular, W of the I-X-W moiety is buried into the protein and oriented between cavity 1 and 2, and the pose is stable in a 30-ns molecular dynamics (MD) simulation. In addition, the ferrocenyl moiety is buried by L369, F382, Y384, K421, Q422, I423, I424, Q428 making a cation-pi interaction with R429. These results provide a framework for optimization of PT entry inhibitors in the context of the trimeric coordinates of HIV-1 Env spike as well as understanding the mechanism of PT binding to HIV envelope protein.

T12. Understanding Dynamic Structural Variations in HIV-1 Envelope Glycoprotein gp120

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HIV-1 envelope glycoprotein gp120 subunits, together with transmembrane glycoprotein gp41, form trimeric spikes on the viral surface. These spikes play a crucial role in initiating HIV-1 infection by binding of gp120 to CD4 receptors and subsequently to co-receptors (CCR5 or CXCR4) on target cells. A full investigation of gp120 dynamics within and between different functional states is of importance to understand protein function and guide vaccine and inhibitor design. To this end, we have performed three independent long-time (1 μs+) molecular dynamics (MD) simulations for the unglycosylated monomeric gp120 core in its free, CD4-bound, and antibody (17b, an antibody to CD4-induced epitopes)-bound states. We studied the dynamics of bound and unbound gp120 core extensively by computing pairwise RMSD matrices, evolutions of secondary structures of each residue, fluctuations, characteristic configurational ensembles and transitions, and global correlated motions from quasiharmonic analysis. We found that the unbound gp120 showed strong conservation of secondary structure, compensating for the fluctuations at the tertiary level. We illustrated a dynamic transition pattern from the CD4 binding interface to opposite side of gp120 with the exception of the terminal β-sandwich during the binding process. A ‘conformational selection’ mechanism of the bridging sheet and ‘induced fit’ mechanism of the CD4 binding site upon receptor association have been revealed. We have reached relative convergence of the unbound trajectory and demonstrated that at least a ms scale of simulation length is needed to equilibrate the gp120 system.

T13. Targeting Cell Surface gp120 with Peptide Triazoles Can Suppress Infectious HIV-1 Formation and Inactivate Virus Producer Cells

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We examined the potential to suppress HIV-1 production from infectious host cells by targeting the Env spike protein. Previous investigation showed that an Env-binding peptide triazole (PT) thiol and its gold nanoparticle conjugate are able to inactivate HIV-1 virions by causing gp120 shedding and lytic release of luminal p24. We asked whether the Env spike expressed on cell surfaces could be disrupted by the PT compounds. Both the PT-thiol KR13 and the gold nanoparticle conjugate AuNP-KR13 suppressed new infectious pseudovirus formation from recombinant 293T cells transiently transfected to produce virus. The budded viruses were inactive, and contained mature p24 capsid protein but not surface gp120. Breadth of virus suppression was demonstrated using 293T cells expressing fully infectious viruses. The peptide triazole compositions also caused shedding from CHO-K1 cells expressing HIV-1 gp120/gp41 complexes. Strikingly, AuNP-KR13 exhibited cytotoxicity against both transfected producer 293T and stably transfected CHO-K1-gp120/gp41 cells, but not untransfected 293T or unmodified CHO-K1 cells. Hence, the peptide triazole class of compounds can impact the HIV-1 life cycle not only by inactivating infectious virions and blocking initial host cell infection, but also by inhibiting new virus production and by inactivating virus-infected cells that express Env gp120 protein on the cell surface. This work demonstrates that cell-surface Env is in a conformationally vulnerable metastable state that is sensitive to inactivation, and identifies a strategy for suppressing latently infected cellular reservoirs of the virus if these can be reactivated to express exposed Env protein.

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BACK

T14. CD4-Env Interaction Stoichiometry Controls the Spatial and Temporal Exposure of the gp41 Trimer

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HIV-1 entry is coordinated by sequential binding of the Env homotrimer to cellular CD4 and chemokine receptors. These events trigger gp41 subunits to transition from the native conformation into a prehairpin intermediate state (PHI) and, ultimately, into a trimer-of-hairpins structure. In the PHI, the gp41 trimer assumes an extended conformation that exposes the N-HR coiled-coil, a target for HIV-1 fusion inhibitors. We investigated the native-to-PHI conformational transition using functionally complemented Env heterotrimers composed of protomers either deficient in CD4-binding (H4-c) or deficient in chemokine receptor-binding (H5). Exposure of the gp41 N-HR region was probed by incorporating fusion-inhibitor escape mutations into individual protomers. The resulting H5 / H4-c heterotrimers contained asymmetric gp41 trimers with both high and low affinity-inhibitor binding sites. We found that inhibitory potency was 20-fold greater when escape mutations were incorporated into H4-c compared to when they were incorporated into H5. This effect was specific for $(H5)_2(H4\text{-}c)$ trimers, which possessed two CD4 binding sites; $(H5)(H4\text{-}c)_2$ trimers, which possessed only one CD4-binding site, exhibited equally low sensitivities regardless of which protomer contained the gp41 escape mutation. The results indicated that the spatial and temporal exposure of the gp41 N-HR coiled coil depends on the number of CD4s that engage the Env trimer. When only one CD4 can bind, the three fusion inhibitor-binding sites on the N-HR coiled coil appear to be exposed symmetrically but only for a short amount of time. By contrast, the binding of two CD4s to the Env trimer appears to reveal a single fusion inhibitor-binding site that remains exposed for a substantially longer period of time. The findings have important implications for the development of vaccine candidates designed to elicit neutralizing antibodies targeting the gp41 N-HR coiled coil. (Funded by NIH R01 GM66682)

BACK

T15. Investigation into the HIV-1 Envelope Lipid-Protein Machine Responsible for the Virolytic Effects of Peptide Triazole Thiols

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HIV-1 can be irreversibly inactivated through shedding of gp120 and lytic release of capsid p24 protein by treatment with peptide triazole thiols (PTTs). The lytic activity suggests that PTT binding to gp120 induces conformational changes in the spike protein and consequent membrane disruption. To investigate the interplay of membrane lipids and spike protein at the virological synapse, the roles of envelope cholesterol and spike gp41 were evaluated.

Cholesterol, which makes up the major lipid constituent (ca. 45 mol %) of the HIV-1 envelope, was investigated for its role in lysis by depletion with methyl beta-cyclodextrin (MβCD). Small amounts of [MβCD] (< 312 μM) led to a pronounced increase in the amount of lysis (*ca*. 2.5x base-line lysis with PTT alone), while greater [MβCD] suppressed lysis. Both the enhancement and suppression of lysis after cholesterol depletion were reversed by replenishment with exogenous cholesterol. Reversal could also be achieved with cholestanol, a sterol that supports lipid raft structures, and to a lesser extent with coprostanol, which does not. Fluorescence measurements at the viral envelope showed a rise in membrane fluidity at > 312 μM MβCD using the probe Laurdan, and a bell-shaped response of fluorescence quenching using the probe dehydroergosterol, with a minimum in intensity at 312 μM MβCD. The specificity of the MβCD-induced effects on lysis was demonstrated by findings that mutations in several membrane-associated domains in gp41, in particular MPER, CRAC and C-terminal tail, caused decreases in the magnitude of the enhancement response. Intriguingly, a bell-shaped response of virus infectivity to MβCD-treatment was observed that was similar to that found for PTT-induced virolysis. These data suggest that virus membrane undergoes morphological changes with the depletion of cholesterol, and that these changes might be linked to both PTT lysis and infectivity pathways.

The overall findings of this work are consistent with the hypothesis that the enhancements of both PTT lysis and infectivity at partial cholesterol depletion are due to lowering the energy barrier for virus membrane disruption. In this view, conformational changes needed to effect virus membrane fusion with the cell membrane or virus membrane lysis with PTTs both require energy, and partial cholesterol depletion from the envelope can reduce the energy required while not disabling the overall activities of lysis and infection. An Arrhenius analysis of this argument, carried out by measuring extents of lysis at different temperatures, showed a reduction of the energy for MβCD-treated virions. Investigation into the mechanism of lysis with PTTs provides a potential window into the mechanism of fusion at the HIV-1 cell interface.

T16. Free-Energy Protocol to Aid the Design Small-Molecule HIV Fusion Inhibitors

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In spite of the attractiveness of HIV gp41 as a viral drug target, there is limited experimental structural information on small-molecule binding, which has made the refinement of reported small-molecule inhibitors challenging. The goal of this work is development of a robust computational protocol, to validate the geometry of ligand-bound structures using as a test case a congeneric series of gp41 inhibitors previously reported by Jiang et al. (*J. Med. Chem.* **2011**, *54*, 572-579) whereby some of the inhibitors' geometries were predicted via molecular docking. In step one of the protocol, the program DOCK is used to generate an ensemble of poses for each inhibitor using receptor-based docking. In step two, the pose ensembles are clustered in order to identify common consensus binding geometries. In step three, the AMBER molecular dynamics (MD) simulation package is utilized to evaluate pose stability with the most stable consensus geometries being used to compute relative free energies of binding between closely-related ligand pairs. Development of protocols to predict ligand-bound geometries and free energies that reflect experimental activity trends will be beneficial in improving the next generation of gp41 inhibitors.

BACK

T17. Highly Potent, Broad Anti-HIV-1 D-Peptide Entry Inhibitor: Development and Preclinical Characterization

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Our lab has developed a potent, protease-resistant D-peptide inhibitor of HIV entry, PIE12-trimer, which targets the highly conserved, transiently exposed gp41 prehairpin intermediate. Although PIE12-trimer potency is impressive (high pM - low nM), its affinity is substantially better (sub-fM). This mismatch is due to the finite exposure of the prehairpin intermediate during HIV-1 entry and the limits of inhibitor diffusion. By "over-engineering" PIE12-trimer with improved affinity, but no corresponding improvement in potency, we create a reserve of binding energy (termed the 'resistance capacitor') that slows the evolution of resistance mutations. To overcome the potency limit, we used membrane-targeting moieties to pre-position PIE12-trimer at the cell membrane, the site of viral entry, thereby facilitating diffusion. We explored a panel of moieties including alkanes, fatty acids, and cholesterol for their effect on PIE12-trimer potency and pharmacokinetics. Cholesterol conjugation improves potency by more than 100-fold, retains an effective resistance capacitor, and substantially increases IV and SC half-lives. The pharmacokinetic profile of chol-PIE12-trimer supports the feasibility of monthly dosing with depot formulation.

T18. Design and Refinement of HIV Inhibitors Using DeNovo DOCK

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In this work we present two uses of a denovo design algorithm currently under development in our group for the program DOCK6: lead refinement and from-scratch construction. In the first set of experiments DeNovo DOCK is used to automate sampling of user-selected "R" groups on a series of HIVgp41 entry inhibitors previously identified through virtual screening. For each hit, several hundred related analogs were generated, many of which show improved compatibility with the known gp41 hydrophobic pocket based on both single point and MD-based energetic analysis. In the second set of experiments, starting from crystallographic structures of HIVPR and HIVRT and libraries of small molecular fragments, DeNovo DOCK was used to construct new molecules into the known binding pockets. As expected, the procedure resulted in a variety of new ligands and promisingly, under certain conditions, known HIVPR and HIVRT inhibitors were also rebuilt. These encouraging preliminary results suggest the algorithm will be a useful tool for design and refinement of new small molecule HIV inhibitors

BACK

T19. Complex Mechanism of Synergy between HIV-1 Entry Inhibitors

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Chemokine-receptor inhibitors (CRI) and gp41 inhibitors block HIV-1 entry through distinct mechanisms. CRIs bind HIV-1 coreceptors on target cells and block their interaction with viral gp120, part of the Env fusion glycoprotein complex. Gp41 inhibitors, also called fusion inhibitors (FI), target a transient intermediate conformation of Env subunit gp41 and block structural transitions required for membrane fusion. Previous work demonstrated that these two inhibitor classes display synergistic activity. The effect is attributed to a reduced rate of coreceptor-triggered conformational transitions in Env, prolonging the intermediate state and, thereby, increasing susceptibility to FIs. Here we show that synergistic activity can be lost in the setting of FI escape mutations that do not disrupt the potency of CRIs, indicating that the mechanism of synergy is more complicated than previously thought. To dissect this mechanism, we employed a series of FIs targeting different regions of gp41 and tested their synergistic activity with CRIs against an array of Env variants. For FIs that target the gp41 N-HR region (T20, C37, PIE12), we show that synergy with CRIs is maximal when the FI binds with extremely high affinity. This synergistic activity is progressively lost as FI binding affinity is disrupted. By contrast, the FI 5-Helix, which targets the gp41 C-HR region, displays a high level of synergy regardless of binding affinity. We correlated these observations with irreversible deactivation of inhibitor-bound gp41. The rate for this deactivation process appears to be dependent on chemokine-receptor binding for N-HR targeted FIs and independent of chemokine-receptor binding for 5-Helix. Finally, using Env heterotrimers with a reduced number of chemokine-receptor binding sites, we show that synergy requires at least two chemokine-receptor binding events per Env complex. The results illuminate the role of chemokine-receptor binding in triggering the intermediate and fusogenic conformations of gp41.

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BACK

T20. Origins of Resistance of T20 with HIVgp41 Using Molecular Dynamics Simulations

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In this study, quantitative results from all-atom molecular dynamics simulations are presented to characterize the binding interactions between the peptide inhibitor T20 and a series of analogs with three variants of the HIV fusion protein gp41. The ultimate goal is to design new fusion inhibitors that can overcome drug resistance through increased understanding of the origins of affinities and mutational effects in the bound complex. Using relative free energy calculations employing the thermodynamic integration (TI) method, the effects of favorable and unfavorable mutations were predicted in reasonable agreement with the experimental trends. Molecular footprint analyses and structural inspections of the endstate complexes were used to identify key interactions between specific residue pairs between T20 and gp41 N-helices, which help explain the underlying effects of primary mutations and secondary mutations for 19 variants of T20 with 3 different gp41 N-helical forms (wildtype, V38A, N43D). In addition, a newly published DOCK scoring function termed pharmacophore matching similarity (FMS) is being employed to help guide virtual screening and *de novo* design targeting gp41 by matching key features in known inhibitors. Preliminary results from FMS-guided modeling will be presented.

T21. Ebselen, a Small Molecule Inhibitor of HIV-1 Capsid Dimerization

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The HIV-1 capsid plays crucial roles in HIV-1 replication and therefore represents an excellent drug target. We developed a time-resolved fluorescence resonance energy transfer (TR-FRET) high-throughput screening (HTS) assay, to screen for inhibitors of capsid dimerization, using the C-terminal domain (CTD) of HIV-1 capsid. This assay was used to screen the Library of Pharmacologically Active Compounds (LOPAC), composed of 1,280 *in vivo* active drugs, and identified Ebselen, an organo-selenium compound, as a potent inhibitor of HIV-1 CTD dimerization. Nuclear magnetic resonance (NMR) spectroscopic analysis confirmed that Ebselen binds directly to the C-terminal domain of capsid. This compound presents anti-HIV activity in single- and multiple- round of infection and displays inhibitory activity in peripheral blood mononuclear cells. It inhibits early post-entry events as shown by the decrease of reverse transcription products and, consequently the number of integrated provirus. These results suggest that Ebselen targets a step prior to reverse transcription, most likely, the uncoating process. Analysis of Ebselen analogues activity suggests that the selenium element of Ebselen is important for its anti-viral activity. Ebselen has also shown to potently block replication of different retroviruses such as Moloney murine leukemia virus (Mo-MLV) and SIVmac239, but displays no inhibitory activity against Hepatitis C and Influenza viruses. This study demonstrates the successful screen of a small molecule library, resulting in the identification of Ebselen as a potent inhibitor of HIV-1 early replication events, reinforcing the notion that HIV-1 capsid is a promising target for drug development.

T22. Live Cell Imaging of HIV Uncoating

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Following the fusion of HIV-1 with the cell membrane the capsid made of p24-CA protein containing viral and host-cell proteins alongside the viral genome is delivered to the cytoplasm. Uncoating is the process of disassembly of the capsid structure and the correct timing of this process has been shown to be vital for successful infection. The kinetics and location of uncoating have been an intense discussion in the recent years. We have recently demonstrated that intravirion content markers, such as free GFP, can be captured within the conical capsid structure. For these studies we utilized the HIV-iGFP construct, where GFP is translated within the interspatial cleavage sites of MA and CA of HIV-1. These viral particles contain free GFP both within and outside the conical capsid structure. The iGFP particles are also labeled with mCherry-Vpr. The intravirion content marker GFP is lost in two steps. First, fusion leads to a drop in GFP signal in particles associated with mCherry-Vpr, and second loss of fluid content GFP marker takes place when the integrity of the conical capsid structure is lost with the initiation of the uncoating process. This makes it possible to identify both the initiation of fusion and uncoating by measuring changes in fluorescence emitted by the individual viral particles over time.

Cells synchronously infected with VSV-G pseudotyped double labeled particles allowed visualization of both the fusion and loss of capsid cone content marker from the mCherry-Vpr labeled complexes. The time lapse observation of mCherry-Vpr labeled complexes revealed the time between fusion and loss of cone integrity was ~30 minutes on average. Interestingly, this timing is consistent with the timing of the CsA washout assay for uncoating as we have previously reported. As seen in the CsA washout assay, we also observed the loss of conical capsid content could be delayed by inhibiting reverse transcription with nevirapine followed by its washout. Cells stably expressing TRIM-CypA revealed that cores are rapidly destroyed after fusion while normal kinetics are measured in the presence of CsA. The addition of MG132 prevents loss of capsid content marker. Importantly, we were able to fix and stain the mCherry-Vpr labeled complexes for the presence of p24 shortly after the loss of capsid content marker. This analysis revealed that the majority of CA protein was lost from the complex. This is consistent with the low levels of CA that we have recently reported are associated with nuclear HIV complexes. This loss of the majority of the capsid reveals the assay is observing uncoating. Importantly, we were able to observe similar kinetics of uncoating after infection of primary macrophages and activated CD4 T cells with infectious virus.

BACK

T23. Single-Molecule FRET Reveals Dynamic Conformational Changes in The Rhesus TRIM5α Dimer

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TRIM5α is a retroviral restriction factor that inhibits infection in a species specific manner. The TRIM5α protein from rhesus macaques (rhTRIM5α) mediates a potent inhibition of HIV-1 infection via a mechanism that involves the abortive disassembly of the viral core. TRIM5α recognition of the viral core is mediated by C-terminal SPRY domains, which potentiate core disassembly via an unknown mechanism. Our recent studies have demonstrated that alpha-helical elements within the Linker2 region, which lies between the SPRY domain and the Coiled-Coil domain, influence the potency of restriction. To understand how these helices affect the structure of the rhTRIM5α dimer, we performed single-molecule FRET analysis on the basic dimeric unit consisting of the Coiled-Coil and Linker2 domains. Studies of the wt dimer revealed three independent FRET states in which the Linker2 termini are displaced by approximately 25 angstroms relative to each other. Additionally, mutations in the L2 region which we have previously found to affect restriction positively or negatively preferentially assumed FRET states occupied by the wt protein but exhibited less frequent transitions between individual FRET states, with restriction defective mutations occupying an extended conformation and restriction enhancing mutations occupying a more compact FRET state. These data suggest a model in which Linker2 mediated displacement of CA bound SPRY domains induces the destabilization of the assembled CA structure during the restriction process.

BACK

T24. RING Dimerization Couples Higher-Order Assembly of TRIM5α to Synthesis of K63-Linked Polyubiquitin

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Members of the tripartite motif (TRIM) protein family of RING E3 ubiquitin (Ub) ligases promote innate immunity responses by catalyzing synthesis of polyubiquitin chains linked through lysine 63 (K63). In this study we investigate the mechanism, by which the TRIM5α retroviral restriction factor activates the ubiquitin conjugate of Ubc13, the K63-linkage specific E2. Structural, biochemical and functional characterization of the TRIM5α:Ubc13-Ub interactions reveals that activation of the Ubc13-Ub conjugate requires dimerization of the TRIM5α RING domain. Our data explain how higher-order oligomerization of TRIM5α, which is promoted by the interaction with the retroviral capsid, enhances the E3 Ub ligase activity of TRIM5α and contributes to its antiretroviral function. The novel E3 mechanism, in which RING dimerization is transient and depends on the interaction of the TRIM protein with the ligand, is likely to be conserved in many members of the TRIM family and may have evolved to facilitate recognition of repetitive epitope patterns associated with infection.

BACK

T25. Asymmetric Conformational Maturation of HIV-1 Reverse Transcriptase

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HIV-1 reverse transcriptase utilizes a metamorphic polymerase domain that is able to adopt two alternate structures that fulfill catalytic and structural roles, thereby minimizing its coding requirements. This ambiguity introduces folding challenges that are met by a complex maturation process. We have investigated this conformational maturation using NMR studies of methyl-labeled RT for the slower processes in combination with molecular dynamics simulations for rapid processes. Starting from an inactive conformation, the p66 precursor undergoes a unimolecular isomerization to a structure similar to its active form, exposing a large hydrophobic surface that facilitates initial homodimer formation. The resulting p66/p66' homodimer exists as a conformational heterodimer, after which a series of conformational adjustments on different time scales can be observed. Formation of the inter-subunit RH:thumb' interface occurs at an early stage, while maturation of the connection' and unfolding of the RH' domains are linked and occur on a much slower time scale.

T26. A Role-Reversal for the p66 and p51 Subunits of HIV-1 Reverse Transcriptase

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HIV-1 reverse transcriptase (RT) is a metamorphic protein in that the sequence does not define a unique fold. The p51 subunit is a truncation of p66, but adopts a different fold and domain arrangement from p66. Typically, when mixing purified p51 with purified p66, the p51 subunit adopts a compact (C) and inactive conformation while the p66 subunit dimerizes with p51 and adopts the extended (E) and polymerase active conformation. In recent experiments we have developed a palm-loop deletion mutant (ΔPL) that fails to isomerize from the compact conformation to the extended conformation. Here we present NMR results demonstrating that p66ΔPL also fails to isomerize from compact conformation even in the presence of p51. Further analysis of the p51 mixed with p66ΔPL shows that the p51 subunit isomerizes to the E conformation and dimerizes with p66ΔPL. This effectively creates a subunit role-reversal in RT such that p51 adopts a conformation containing an active polymerase site and p66ΔPL adopts the compact conformation that fulfills a structural role in the dimer. The results demonstrate effective subunit and conformation specific labeling. The approach has been further extended to study ^{15}N-^{2}H uniform labeling of the p66ΔPL monomer for comparisons with RT. The results highlight the asymmetric and metamorphic nature of RT. Understanding the many different conformations that the protein can adopt may facilitate the development of conformationally-targeted drugs.

T27. Structure of HIV-1 Reverse Transcriptase Bound to a Novel 38-mer Hairpin Template-Primer DNA Aptamer

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The development of a modified DNA aptamer that binds HIV-1 reverse transcriptase (RT) with ultra-high affinity has enabled the X-ray structure of an HIV-1 RT-DNA complex to 2.3 Å resolution without the need for an antibody Fab fragment or RT-DNA cross-linking. The 38-mer hairpin-DNA aptamer has a 15 base-pair duplex, a three-deoxythymidine hairpin loop, and a five-nucleotide 5'-overhang. The aptamer binds RT in a template-primer configuration with the 3'-end positioned at the polymerase active site and has 2'-O-methyl modifications at the second and fourth duplex template nucleotides that interact with amino acid residues in the p66 fingers and palm subdomains. This structure represents the highest resolution RT-nucleic acid structure to date. The RT-aptamer complex is catalytically active and can serve as a platform for studying fundamental RT mechanisms and for development of anti-HIV inhibitors through fragment screening and other approaches. Additionally, the structure allows for a detailed look at a unique aptamer design and provides the molecular basis for its remarkably high affinity for RT.

T28. Cryo-Electron Microscopy of Retroviral Initiation and Integration Complexes

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Single particle cryo-electron microscopy (cryoEM) enables structural characterization of large (>100 kDa) macromolecules and macromolecular complexes. Recent technological improvements have provided near-atomic resolution density maps of select macromolecular complexes, typically ones that are large, and which therefore contain sufficient signal. Several of the core aims of the HIV Interaction and Viral Evolution (HIVE) P50 center revolve around the structural and functional characterization of key components within the HIV lifecycle, including the retroviral initiation complex and the retroviral integration complex. We are using single-particle cryoEM to decipher these key structural components and present current progress of cryoEM structural analysis at the HIVE center.

T29. Subunit-Specific ^{15}N-Isotopic Enrichment of HIV-1 Reverse Transcriptase Facilitates Unambiguous Peptide Identification During Hydrogen-Deuterium Exchange Coupled to Liquid Chromatography-Mass Spectrometry (HDX-MS): Applications to Drug Binding and Mapping Nucleic Acid Contacts

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Hydrogen-deuterium exchange coupled to liquid chromatography and mass spectrometry is a useful method for exploring protein-ligand, protein-protein, and protein conformational dynamics in solution. HIV-1 reverse transcriptase is a multifunctional and highly dynamic enzyme that interacts with double stranded DNA and RNA, small molecule substrates, and antiretroviral drugs. Crystallographic fragment screening in our lab has shown that RT is highly mutable and accommodates multiple fragment-induced binding pockets.

RT's importance as a drug target makes it a good candidate for study by HDX-MS for conformation dynamics, ligand binding, and mapping nucleic acid contacts. However, RT poses a special challenge for study by HDX since it is composed of two subunits, 66 and 51 kDa (p51 and p66) that form the RT heterodimer. The p51 subunit shares 440 residues with p66 making it impossible to determine from which subunit a peptide is derived during the HDX experiment.

We used ^{15}N-isotopic labeling of the p51 subunit to unambiguously assign peptides to p51 or p66. This method was employed to study binding of the NNRTI drug efavirenz and to map contacts between RT nucleic acid substrates. Our results from EFV binding to RT are in agreement with previously published studies: RT binding to EFV results in an overall decrease in exchange from D→H with the greatest protection observed for RT residues in the EFV binding pocket. For studies with RT and nucleic acids we found that regions of RT that are protected in the presence of duplex DNA are in good agreement with known contacts observed in RT-DNA crystal structures. We also show that HDX-MS on a model of the RT initiation complex made up of RT bound to host-cell $tRNA^{lys,3}$ and 77 nucleotides of the viral genomic (PBS) region make contacts beyond the RT-DNA envelope that are in agreement with previous crosslinking studies on a model of the HIV initiation complex.

BACK

T30. Effect of Nucleic Acid Sequence on the DNA Polymerization and NNRTI Inhibition Mechanisms of HIV-1 Reverse Transcriptase

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HIV-1 reverse transcriptase (RT) is a well-studied enzyme and the target of most approved antiretroviral drugs. Nonnucleoside RT inhibitors (NNRTIs) are clinically important for the treatment of HIV infection. Numerous biochemical and structural studies have provided valuable insights into the mechanisms by which NNRTIs affect the activity of HIV-1 RT.

Structural comparisons of RT in complex with various NNRTIs have revealed differences in the conformation of DNA-interacting structural regions of RT, such as the p66 thumb and the DNA primer grip. Moreover, a recent crystal structure of RT in complex with DNA and nevirapine (Das *et al.* NSMB, 19, 253) has shown that NNRTI binding repositions the 3' end of the DNA primer and distorts the dNTP-binding site that comprises protein as well as DNA components. Hence, we hypothesized that variations in DNA sequence can modulate the efficacy of NNRTIs and their mechanism of inhibition.

To test this hypothesis, we determined the RT position on DNA of various sequences using a site-specific hydroxyl-radical footprinting assay in the presence and absence of different concentrations of various NNRTIs. This approach revealed surprising changes in RT-DNA binding caused by different NNRTIs, with lateral displacements of template/primers that were dependent on the nucleic acid sequences as well as on the type of NNRTI bound.

To determine whether these site-specific changes correlate with differences in the ability of RT to biochemically recognize the dNTP and DNA substrates, we performed transient-state kinetic analysis and demonstrated significant sequence-specific changes in DNA- and dNTP-binding affinities ($K_{D.DNA}$ and $K_{D.dNTP}$ respectively) and catalytic turnover (k_{pol}).

Taken together, our data suggest that (a) NNRTIs have significant effects on RT-DNA binding conformations, which are greatly influenced by the DNA sequence and the type of NNRTI, and that (b) the DNA polymerization properties of RT vary significantly with DNA sequence.

T31. Rilpivirine Resistance in HIV-1 Subtype C from Low and Middle Income Countries

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Second generation non-nucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (RPV) has been co-formulated with tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) as a single tablet regimen (STR), and has been recommended for patients with viral load <100,000 copies/ml in resource-rich countries. Due to its ability to inhibit viruses that are resistant to 1st-generation NNRTIs nevirapine and efaverinz, RPV use is also under consideration in low- and middle- income countries (LMICs). We have analyzed a dataset of nearly 20,000 patients from high-income European settings and two LMICs, India and Ethiopia to determine feasibility of RPV use among patients infected with HIV subtype C (HIV-1C), since HIV-1C is most dominant in these LMICs. We analyzed the reverse transcriptase (RT) sequences from three clinical trials: (i) therapy-naïve individuals were from the Swedish InfCare cohort (n=4597), India (n=617) and Ethiopia (n=126), (ii) patients (n=117) in Swedish InfCare who were initiated on RPV-based therapy and followed up for 96 weeks, and (iii) patients who failed 1st-generation NNRTIs in EuResist database (n=13750). We searched for the presence of mutations that have been associated with RPV resistance in clade B, as markers of potential RPV resistance in other HIV clades (nonB). The potential of Primary RPV resistance or cross-resistance with other NNRTIs was assessed by Rega (v9.1.0) and Stanford HIVDB (ver7.0.1). We find a low-level of primary RPV resistance mutations in treatment-naïve patients in all subtypes. However, cross-resistance to RPV was notably higher in HIV-1C than in HIV-1B patients ($p<0.001$). Failure of RPV-based therapy was greater in HIV-nonB (~25%) than in HIV-1B patients (9%) ($p=0.03$). We also cloned, expressed, and purified RTs from patients infected with non-B HIV, and assessed *in vitro* RPV susceptibility and generated molecular models to help interpret biochemical and clinical data. *In vitro assays* showed higher RPV IC_{50} values (~2 fold) for HIV-1C RTs compared with HIV-1B RTs ($p<0.025 – 0.05$). We conclude that (i) RPV may have suboptimal effect in HIV-1C dominated epidemics as a first-line drug due to a high proportion of treatment-naïve individuals with a high viral load, and (ii) it is feasible to use RPV in second-line or as alternate regimen if pre-therapy genotypic resistance testing is performed.

T32. Structural Integrity of the Ribonuclease H Domain in HIV-1 Reverse Transcriptase: pH Dependence Study

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Reverse Transcriptase (RT) of Human Immunodeficiency Virus type 1 (HIV-1) is encoded as a 66 kDa protein in the virus, and is matured to form a heterodimer composed of 66 kDa (p66) and 51 kDa (p51) subunits. The p51 subunit is generated by proteolytic cleavage of the most of the ribonuclease H domain (RNH) from a p66 protein by HIV-1 protease. Interestingly, the p66 homodimer is also known to be enzymatically active *in vitro*. However, mutagenesis of the RT p51↓RNH cleavage-site sequence is known to result in significantly attenuated infectivity and abnormal proteolytic processing of RT (1). Further study led to the identification of a compensatory mutation, T477A, which restores proteolytic stability of p66 in the RT p51↓RNH mutants (2). The structural basis for the observed differences in proteolytic stability of the RT p51↓RNH mutants and the T477A revertant mutants is unknown. We are characterizing conformation of RNH domains with these cleavage-site mutations and the revertants using experimental and computational methods. We present the progress of this study with emphasis on the pH dependent protein folding of these mutants.

Support: the National Institutes of Health P50GM082251 and P50GM103368.

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2. Abram, et al. 2010. Retrovirology. 7:6

BACK

T33. Structural Features of the HIV-1 Reverse Transcriptase RNase H Domain Reveal a Propensity for Domain Swapping And Unfolding

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Formation of the mature HIV-1 reverse transcriptase p66/p51 heterodimer requires domain specific processing of the RH domain. Since the RH domain contains an occult cleavage site located near the center of the domain, cleavage must occur either prior to folding or subsequent to unfolding. Recent NMR studies provide substantial support for a subunit-specific unfolding process, starting from a p66/p66' homodimer that exists as a conformational heterodimer. Tyr427 and other residues at the N-terminus of the initially folded RH' domain are transferred to the C-terminus of the polymerase' domain of p66'. However, the molecular basis for this process remains undetermined. In the present study, analysis of the RH domain swapped dimer provides important insight into the unfolding process that has direct implications for RH unfolding. Variable positioning of helix B, due to a lack of stabilizing sidechain interactions, is structurally coupled to the flexibility of the hydrophobic pocket containing Tyr427, facilitating occasional release of the Tyr427 sidechain. In the domain-swapped dimer, aD is extended and the B-D hinge loop that connects the two monomers is significantly shorter than the monomer B-D loop. Shortening of the hinge loop in domain swapped structures is a common observation, interpreted to indicate that a sub-optimal length of the loop acts as a destabilizing feature of the monomer structure. These characteristics facilitate occasional loss of Tyr427 from the globular RH domain that is present in the p66 monomer. After initial formation of the p66/p66' dimer, unfolded Tyr427 can be recruited into helix aM in the polymerase domain, limiting the rate of reverse transfer and leading to further residue transfer, RH domain destabilization, and unfolding.

T34. Conformational Responses of RNase H of HIV-1 Reverse Transcriptase upon Inhibitor or Ligand Interaction.

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HIV reverse transcriptase (RT) is a multifunctional enzyme with both DNA polymerase and ribonuclease H (RNH) activities and is essential for HIV replication. Although many inhibitors against the polymerase activity have been developed, there is no clinically approved drug that inhibits the RNH activity of HIV. In contrast to the 20-plus years of study on the mechanisms of polymerase inhibitor interaction on RT, structural information for RNH inhibitor (RNHI) interactions is scarce. Only recently were several crystal structures of the RNH active-site inhibitors in complex with RT or the RNH fragment published, and no structural analysis of non active-site RNHI has been reported. Thus, we are characterizing the interaction of the inhibitor with RNH and RT by combined use of Nuclear Magnetic Resonance (NMR) spectroscopy and biochemical methods. We are also revisiting the RNH conformational changes upon Mg^{2+} interaction, with increased sensitivity, by NMR.

BACK

T35. Re-Purposing HIV-1 Reverse Transcriptase-Associated Ribonuclease H Inhibitors.

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The absolute requirement on reverse transcriptase-associated ribonuclease H activity for HIV replication has promoted several drug discovery efforts to target this C-terminal domain of its p66 subunit, none of which have advanced to clinical trials. Since HIV RNase H is one of >100 members of the nucleotidyl phosphotransferase (NPTase) superfamily, we asked whether active site HIV RNase H inhibitors could be re-purposed to target NPTases of other viruses with which HIV infection is associated. In particular, herpes simplex virus type 2 (HSV-2) infection has been shown to facilitate the risk of HIV acquisition and often worsen the clinical course of the HIV disease. As cells of the monocyte/macrophage lineage reside in genital mucosal tissues are thought to be reservoirs of HIV-1, developing an HIV-1/HSV-2 microbicide combination would clearly be beneficial.

UL15 is a component of the HSV terminase molecular motor, whose C-terminal nuclease domain is proposed to use an RNase H-like, 2-metal ion-mediated catalysis mechanism for cleavage and packaging of its concatemeric DNA genome. The crystal structure of this C-terminal domain (designated pUL15C) was recently solved, strengthening the notion that this HSV NPTase would a candidate for inhibition by a-hydroxytropolone-derived HIV RNase H inhibitors. Since the HSV encapsidation machinery has no counterpart in the mammalian cell, targeting encapsidation presents an attractive antiviral strategy. Using differential scanning fluorimetry (DSF, or Thermofluor), we showed that a-hydroxytropolone binding to HSV pUL15C resulted in stabilization against thermal denaturation by as much as 9°C. A series of model duplexes was used to investigate the specificity of pUL15C cleavage, from which we developed a novel fluorigenic substrate for high throughput screening. Data with this Q670/BHQplus system showed a direct correlation between pUL15C stabilization against thermal denaturation and inhibition of nuclease activity, yielding novel chemotypes with IC_{50} values of ~150nM from our initial screen. Parallel *in vivo* studies have shown that a-hydroxytropolones inhibit replication of both wild type and acyclovir-resistant HSV-1 and HSV-2, while time-of-addition studies suggests inhibition at different stages of virus replication. Since the terminase molecular motor is common to human cytomegalovirus (HCMV) and Kaposi's sarcoma herpvesvirus (KSHV), re-purposing small HIV RNase H inhibitors to target related NPTases illustrates a novel strategy to combat HIV-associated co-morbidities with a viral etiology.

BACK

T36. CBF-β Dispensability for Non-Primate Vif Function Hints at HIV-1 Accessibility to Alternative Routes Through Cellular Pathways

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HIV-1 replication essentially utilizes host cell machinery, although we suggest that this critical reliance is at a pathway level such that virus adaptation may exploit cellular redundancies to substitute favored specific host-virus interactions when blocked by antiviral or cellular challenges. While the high mutational rate of viruses affording emergent resistance to traditional drugs targeting viral proteins has driven a shift in therapeutic strategies towards targeting key host-virus interactions, we suggest that a new kind of viral resistance may emerge whereby competent viruses may completely reroute to utilize alternative host domains available within the same cellular pathway. We rationalize that the limited overlap of host factors identified in large-scale interactome studies as vital for HIV-1 replication can be explained by considering that HIV-1 target, rather than specific factors, crucial cellular pathways using different protein-protein interactions within that pathway.

As a detailed proof of principle and to demonstrate the significance of viral rerouting we are characterizing the rerouting landscape around the critical HIV-1 Vif–human CBF-β interaction. Whereas CBF-β is essential for Vif orchestrated propeosomal degradation of APOBEC3s in HIV-1, it has been reported dispensable for Vif function in non-primate lentiviruses. The cross-species nuances in Vif biochemical and functional features may indeed highlight conceivably latent escape mechanisms accessible to challenged HIV-1 Vif and emphasize the urgency in recognizing the importance of viral flexibility in pathway rerouting as a pathogenic survival tactic.

BACK

T37. Vif-Specific Fab Inhibits Ubiquitination and Degradation of A3F

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The severe pathogenesis associated with HIV is due in large part to potent inhibition of host immune mechanisms. Among the known lentiviral accessory proteins, the virally encoded Vif protein plays an important role in counteracting the antiviral effects of host APOBEC3 innate immune proteins. Vif antagonizes APOBEC3 by hijacking a cellular Cullin-RING ubiquitin ligase, resulting in the ubiqutination and subsequent targeting of APOBEC3 for proteasomal degradation. Vif-mediated inhibition of APOBEC3 is critical to lentiviral pathogenesis, therefore, Vif is a promising therapeutic target which may lead to novel anti-HIV strategies. In order to disrupt the Vif-A3 interaction and validate Vif as a therapeutic target, we generated Fabs against the HIV-1 Vif/EloB/EloC/CBF-β complex and tested their ability to inhibit APOBEC3 ubiquitination. Here we show that our selected Fabs bind to the HIV-Vif complex with high affinity and are capable of inhibition Vif-mediated A3F degradation. Using our *in vitro* ubiquitination assay, we established that one of our identified fabs, termed 3C9, successfully inhibited A3F-CTD ubiquitination. Consistent with the ability to antagonize the A3F-CTD poly-ubiquitination *in vitro*, a single chain variant of 3C9 can inhibit Vif-mediated A3F degradation in celluo, and importantly can promote A3F packaging into virion. Taken together, our results support the identification of a Vif-specific Fab that binds the HIV-1 Vif complex with high affinity and specificity, and has the capacity to potentially function as a Vif-E3 ligase inhibitor. These findings validate Vif as a promising therapeutic target, and for the first time experimentally establish the feasibility of generating inhibitors that directly target Vif, and specifically the Vif-A3 interface.

T38. Human Restriction Factor and Genomic DNA Modifier APOBEC3B: NMR Structure, Substrate Binding and Deaminase Activity

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Human APOBEC3B (A3B) is one of seven APOBEC3 family members, which are single-stranded DNA cytidine deaminases that restrict viral pathogens and endogenous retrotransposons and play a role in the innate immune response. A3B is unique since it is the only member of the family that is exclusively localized to the cell nucleus. Recently, A3B was found to be a major source of genetic heterogeneity in several human cancers. To gain insights into A3B function, we purified the active C-terminal deaminase domain of A3B (A3B-CTD) from *E. coli* and carried out a structural and biochemical analysis. Size exclusion chromatography together with multi-angle light scattering (SEC-MALS) showed that the protein exists as a monomer in solution. Further, the purified A3B-CTD exhibited a well dispersed ^{1}H-^{15}N HSQC spectrum. Complete ^{1}H, ^{13}C and ^{15}N NMR assignments were obtained and the high-resolution NMR structure of A3B-CTD was determined. The A3B-CTD structure shows a central five-stranded β-sheet with six surrounding helices, a conformation common to all APOBEC3 proteins, with A3B-CTD most closely resembling the A3A and A3G-CTD structures. Multiple conformations undergoing motions on a micro-millisecond time scale were observed for loop 1 and loop 3. Deamination real-time kinetics studies, conducted by NMR under various conditions, revealed that A3B-CTD is a weaker deaminase (~30-fold) than A3A. The in vitro enzymatic activity of the CTD (assayed in 293T cell extracts) was found to be ~2-fold lower than that of the full-length protein, although the nucleotide substrate preferences were the same. In summary, structural mapping of the DNA binding site and deamination kinetics provide invaluable information on the structural basis for the different activities of the APOBEC3 proteins.

BACK

T39. Assembly and Characterization of the HIV Vif E3 Ligase with Full-Length Human APOBEC3G

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The APOBEC3 family of restriction factors protects hosts from retroviruses and retroelements. They are polynucleotide cytosine deaminases that restrict by mutating viral cDNA. The lentiviral Vif protein promotes spread of virus in host by targeting APOBEC3 family members for degradation by the 26S proteasome. To do so, Vif must hijack an E3 ligase from the Cullin-RING super family. In primates the action of the Vif-E3 requires an accessory subunit, core-binding factor beta. Structural studies of full-length human APOBEC3G in complex with Vif-E3 is a challenge for the field. Here we report the reconstitution and characterization of full-length human APOBEC3G with the HIV-1 Vif holoenzyme. A protocol for preparing multi-mg quantities of full-length human APOBEC3G in complex with the Vif-E3 is presented. The particle is monodisperse. The stoichiometry of the APOBEC3G and the Vif-E3 is revealed by SEC-MALLS. Separation of function mutants known to block Vif neutralization of A3G and A3F in cells also impair polyubiquitination activity of Vif in vitro. These results indicate defined complexes between the Vif-E3 and APOBEC3G can be prepared in large quantities in a form that is suitable for structural analyses.

T40. Structure of the Vif-Binding Domain of the Antiviral Enzyme APOBEC3G

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The human APOBEC3G (A3G) DNA cytosine deaminase restricts and hypermutates DNA-based parasites including HIV-1. The viral infectivity factor (Vif) prevents restriction by triggering A3BG degradation. Although the structure of the A3G catalytic domain is known, the structure of the N-terminal Vif-binding domain has proven more elusive. Here we used evolution- and structure-guided mutagenesis to solubilize the Vif-binding domain of A3G, thus permitting structural determination by NMR spectroscopy. A smaller zinc-coordinating pocket and altered helical packing distinguish the structure from previous catalytic-domain structures and help to explain the reported inactivity of this domain. This soluble A3G N-terminal domain is bound by Vif; this enabled mutagenesis and biochemical experiments, which identified a unique Vif-interacting surface formed by the α1-β1, β2- α2 and β4- α4 loops.

This structure sheds new light on the Vif-A3G interaction and provides critical information for future drug development.

T41. Interactions Between APOBEC3 and Viral and Cellular RNA

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APOBEC3 proteins represent a class of restriction factors that, in the absence of Vif, are able to modify cytosines to uracils in single-stranded DNA retroviral replication intermediates, resulting in lethal hypermutation of the viral genome. APOBEC3 proteins have also been proposed to inhibit viral replication in a manner that is independent of deaminase activity. In order for APOBEC3 proteins to exert an antiviral effect they must be incorporated into nascent virions in an RNA-dependent manner by binding to viral or cellular RNAs.

To probe the molecular interactions between APOBEC3 proteins and viral and cellular RNA we employed cross-linking immunoprecipitation sequencing (CLIP-seq) of APOBEC3F, APOBEC3G and APOBEC3H proteins in HIV-1 infected and uninfected human cells and in virions. We found that APOBEC3 proteins bind to several classes of RNAs in infected and uninfected cells and virions, including cellular messenger RNAs, genomic viral RNA and non-coding RNAs, such as rRNAs, tRNAs and 7SL RNA. The bulk of RNA that was bound by APOBEC3 proteins in both infected and uninfected cells was cellular mRNA, with genomic viral RNA representing a major class of bound RNA in infected cells. In HIV-1 virions, the majority of RNA bound by APOBEC3 was found to be genomic viral RNA. Alignment of RNA sequences that were bound by APOBEC3 in cells to the HIV-1 genome revealed that the 3′ portion of the viral genome was extensively occupied by APOBEC3, with discrete sequences containing a significant number of reads. Binding to the 5′ portion of the viral genome, however, was drastically reduced. In virions, the occupancy of APOBEC3 was more evenly distributed along the length of the viral genome, with significant numbers of reads mapping to discrete sequences. We used evidence-ranked motif identification to obtain representations of preferred sequences that are bound by APOBEC3 proteins. This analysis revealed that APOBEC3H has a preference that is distinct from that of APOBEC3F and APOBEC3G. Overall, these data reveal the molecular details of how binding to RNA drives APOBEC3 incorporation into virions.

BACK

T42. Structural and Specificity Studies of APOBEC3 Domains

Tania V. Silvas[1], Shivender M.D. Shandilya[1], Markus-Frederik Bohn[1], Shurong Huo[1], Ellen A. Nalivaika[1], Takahide Kouno[2], Nese Kurt-Yilmaz[1], Mohan Somasundaran[3], Reuben S.Harris[2], Matsuo Hiroshi[2], and Celia A. Schiffer[1]

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Our project team has determined the experimental structures of four APOBEC3 domains and modeled the remaining domains. These structures, complemented with ssDNA binding assays, begin to provide us insights into how these domains recognize specific substrates, bind HIV-1 Vif and oligomerize, and through detailed structural comparisons we begin to gain insights into the molecular mechanisms by which these enzymes distinguish their functions and can potentially be specifically targeted by therapeutics.

T43. Editing Editors with Editing

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The APOBEC3 family of DNA cytosine deaminases is an important part of the innate immune system. These proteins help protect humans from retrovirus and retrotransposon replication. For example, many studies over the past decade have demonstrated that APOBEC3D, APOBEC3F, APOBEC3G, and APOBEC3H combine to restrict HIV-1 by a cDNA cytosine deamination mechanism (1). To counteract restriction by these APOBEC3s, HIV-1 produces a protein called Vif, which mediates APOBEC3 degradation through a polyubiquitination and proteasome degradation mechanism (1). The physiological balance between APOBEC3 restriction and Vif counteraction is not absolute because HIV-1 sequences from patients often bear APOBEC3 signature G-to-A mutations. We hypothesize that APOBEC3 proteins provide a dominant contribution to the overall HIV-1 mutation rate (*i.e.*, greater than reverse transcriptase). To test this hypothesis, we used the CRISPR/Cas9 editing system to delete the entire 120kb *APOBEC3* locus from CEM2n, which is a near-diploid $CD4^{+}$ human T-cell line that supports HIV-1 replication and expresses multiple restrictive APOBEC3 proteins (Refsland *et al.*, 2012, *PLoS Pathogens*). *APOBEC3* null clones have been identified by PCR and immunoblotting, and will be used for virus replication and mutagenesis experiments. Results and observations to date will be presented.

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BACK

T44. Progress on Developing Small Chemical Inhibitors of the APOBEC3 Family of DNA Cytosine Deaminases

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APOBECs are a family of nine active DNA cytosine deaminases with a variety of biological roles in human cells, including retrovirus and retrotransposon restriction, foreign DNA restriction, and antibody gene diversification (1). To develop chemical probes for these enzymes, we optimized a fluorescence-based single-strand DNA deaminase assay and used it to screen over 500,000 compounds for enzyme inhibition. Parallel screens were done using APOBEC3A/APOBEC3B and APOBEC3G to help distinguish specific and broad-spectrum chemical inhibitors. These screens have yielded over 2,300 primary hits with various specificities and chemical scaffolds. Biochemical and structural studies demonstrate several distinct mechanisms of inhibition (*e.g.*, 2, 3). Screening data and progress on scaffold validation will be summarized.

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T45. Structural and Functional Characterization of The Mouse Mammary Tumor Virus Intasome

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Retroviral integrase (IN) mediates the integration of the viral reverse transcript into host DNA, an essential step in the retroviral replication cycle. The active IN nucleoprotein complex, comprised of IN bound to its cognate viral DNA, is called the intasome. To date high resolution structures are available only for the prototype foamy virus (PFV) intasome. These advances are in large part due to favorable PFV IN biochemical properties, including highly soluble protein, and the ability to efficiently integrate two surrogate viral DNA ends into target DNA *in vitro*. The intasome structures provide unprecedented details on the molecular mechanism of retroviral DNA integration as well as the mechanisms of action of clinical strand transfer inhibitors. PFV nevertheless represents just one genus of retrovirus. Additional intasome structures will accordingly increase the breadth of knowledge of retroviral integration.

highly active intasomes *in vitro*. Intasome-mediated concerted viral DNA integration yielded a 6 bp duplication of target DNA, which is known to occur during MMTV infection. MMTV intasomes were stable for up to three weeks at 4 ºC and were inhibited by HIV-1 IN strand transfer inhibitors.

The MMTV intasome was analyzed using single-particle cryo-electron microscopy, which yielded a 6.8 Å resolution map for the protein-DNA complex. MMTV IN is composed of the three canonical IN domains – the N-terminal domain, catalytic core domain (CCD), and C-terminal domain (CTD) – and homology models of the different domains were built using the structures of Rous sarcoma virus and HIV-2 IN domains as templates. Placement of the domains into the EM map by rigid body docking indicates that the MMTV intasome is composed of an octamer of IN – four dimers of full-length IN – in complex with two molecules of viral DNA.

The novel octameric architecture of the MMTV intasome was compared with the known tetrameric IN architecture of the PFV intasome, which revealed a common intasome core structure composed of four IN molecules and two viral DNA ends. Our data indicates that the length of the linker connecting the CCD and the CTD, which is relatively short for MMTV IN, is the main factor that dictates the stoichiometry of IN-to-DNA among the retroviral intasomes.

T46. Modeling Molecular Recognition by HIV-1 Integrase and Protease Using Binding Free Energy Methods

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In recent years, allosteric HIV-1 integrase inhibitors (ALLINIs) have emerged as a promising new class of antiviral compounds (1,2). ALLINIs bind at the catalytic core domain (CCD) dimer interface in the LEDGF/p75 binding pocket. Recent studies have shown that ALLINIs primarily function by promoting aberrant, higher-order multimerization of IN during virus maturation resulting in non-infectious particles. In collaboration with the labs of Kvaratskhelia and Olson, we have been employing binding free energy methods (BEDAM, DDM and FEP) to develop more potent ALLINIs, and to determine the basis for drug resistance mutations towards ALLINI (3,4).

In the first part of this presentation we applied Free Energy Perturbation (FEP) methods to predict the binding affinities towards CCD for a series of pyridine based compounds derived from a known multimerization specific inhibitor KF115. These derivative compounds contain varying substituent groups attached to the pyridine core: R1, R2 and R3. Experimentally R3 is found to be key to promote multimerization. Free energy calculations dissected the contributions of different R-groups to the binding with the CCD dimer. While R1 and R2 are found to be essential to binding with CCD, R3 contributes very little to CCD binding. This suggests that R3 is likely to play the role of engaging other subunits in multimerization. Our calculations have identified several promising R1 variants with better or comparable binding affinities, compared with KF115. One predicted top binder (compound 233) utilizes a pyrimidine moiety at R1 to form a hydrogen bond with Thr125. Calculations on a series of R2 variants show that substituting the t-butyl ether at the R2 position leads to generally weaker binding compared with KF115.

In the second part of the presentation, we discuss our recent methodology development to improve predictions of the binding pose and binding free energy. We first show that a new method of binding energy landscape analysis can lead to improved enrichment in virtual screening. In the SAMPL4 blind challenge for predicting IN-small molecule binding, 80% of the false negatives are associated with bad initial docked poses. Revisiting these ligands retrospectively using BEDAM (5) shows that the majority of them have a funneled binding energy landscape favoring the crystallographic binding pose. Based on this analysis, we have developed a procedure to combine binding energy landscape analysis with docking to more reliably identify binders in virtual screening experiments.

We also describe a new sampling method for binding free energy simulations. Currently, accurate binding free energy calculations rely on having the correct initial structure of the complex, because free energy simulations will usually be trapped in the neighborhood of the starting conformation. This has limited the applications of binding free energy methods in drug discovery. Our new sampling method is based on BEDAM (5) and uses torsional flattening within a Hamiltonian replica exchange scheme to overcome this limitation and remove the dependence on the initial structure. This method is showing promise in a set of 27 false negatives of HIV-1 IN LEDGF site ligands taken from SAMPL4: Starting from an incorrectly docked structure, the crystallographic binding poses are recovered in ~ 50 % of the false negatives.

Lastly, we describe our free energy simulations of the exo-site of HIV-1 protease (PR). Ligand binding at this site was found to weakly inhibit HIV-1 PR. A series of ligands were design using docking and pharmacophore methods based on the original weak inhibitor. We have preformed molecular dynamics and free energy simulations starting from the docked structures to explore the binding modes and binding affinities of the designed ligands. The results show that the computed binding affinities of the new ligands are stronger compared with the known inhibitor and provided insights into the stabilities of the binding modes of the designed inhibitors. Currently, NMR labeling experiments are being conducted to test and validate these results.

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BACK

T47. Molecular Dynamics Studies of ASV and HIV Integrase Reaching Dimers

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We have shown that retroviral integrases are able to form alternate dimer assemblies. One, termed a core-core dimer, is stabilized predominantly by an extensive interface between two catalytic core domains. A second, called a reaching dimer, is stabilized by N-terminal domains (NTD) that reach to form intermolecular interfaces with the other subunit's core and C-terminal domains (CTD), as well as CTD-CTD interactions.

In this study, molecular dynamics (MD), Brownian dynamics (BD) simulations, and free energy analyses, were performed to elucidate determinants for the stability of the reaching dimer forms of full-length ASV and HIV IN, and to examine the role of the C-tails (the last ~16-18 residues at the C-termini) in their structural dynamics.

The dynamics of an HIV reaching dimer derived from SAXS and protein crosslinking data, was compared with the dynamics of a composite, core-core dimer model derived from combining the crystal structures of two-domain fragments. The results showed that the core domains in the ASV reaching dimer express free dynamics, whereas those in the HIV reaching dimer are highly stable. Brownian dynamics simulations suggest a higher rate of association for the HIV core-core dimer than the reaching dimer. The predicted stability of these dimers was therefore ranked in the following order: ASV reaching dimer<HIV reaching dimer<composite dimer. Analyses of MD trajectories have suggested residues that are critical for intermolecular contacts in each reaching dimer. Tests of these predictions and insights gained from these analyses could reveal a potential pathway for the association and dissociation of full-length IN multimers.

BACK

T48. Visualizing HIV-1 Integration

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The sequence of events from HIV-1 cytoplasmic entry until integration of proviral DNA is not well understood. While many studies have attempted to investigate these post-fusion entry events, the unstable nature of intracellular HIV particles has made biochemical analyses difficult. Here, we developed a system to visualize HIV viral DNA by incorporation and subsequent click-chemistry labeling of the thymidine analog 5-ethynyl-2'-deoxyuridine (EdU) into nascent HIV reverse transcription (RT) products. To label HIV viral DNA in vivo, we infected monocyte-derived macrophages (MDM) cultured in the presence of EdU. Because MDMs are non-dividing primary cells they do not incorporate EdU into their chromosomes, thus allowing detection of intranuclear HIV genomes. Using this technique, we observed accumulation of EdU punctae in the cytoplasm and nucleus of infected MDMs that remained stable through 5 days post-infection. Knockdown of SAMHD1, a dNTPase that maintains low nucleotide pools in myeloid cells, using Vpx-containing SIV virus-like particles (VLPs) resulted in a markedly increased number of EdU punctae that correlated with the percentage of productively infected cells. Importantly, inclusion of RT inhibitors blocked HIV EdU formation whereas integrase inhibitors did not, indicating that EdU incorporation is dependent on reverse transcription of the HIV genome but not on chromosomal integration. Using fluorescence in situ hybridization (FISH), we confirmed that the cytoplasmic and nuclear EdU punctae contained HIV DNA sequences, indicating that EdU is a reliable and specific marker of synthesized HIV viral DNA. Interestingly, we detected EdU signals in MDMs that were productively infected as well as in cells that were not producing HIV proteins, suggesting that the technique can detect latent or abortive infections on a single cell level. In some cells, EdU colocalized with γ-H2AX, a histone protein that is heavily phosphorylated at sites of DNA double strand breaks, suggesting that these punctae were sites of integrated HIV. We also used RNA FISH to identify integrated and actively transcribing HIV proviruses by detecting colocalization of EdU punctae with HIV RNA sequences. EdU labeling, therefore, is useful for tracking the fates of viral DNA within the cytoplasm and nuclei of infected cells and provides a reliable marker of HIV integration sites in productively infected primary macrophages.

T49. Imaging Studies Demonstrate that Trafficking of HIV-1 Pre-Integration Complexes into the Nucleus Requires TNPO3 and Nuclear CPSF6 Expression

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Early post-entry steps of HIV-1 infection are poorly understood. Previous studies have shown that expression of transportin 3 (TNPO3), which is nuclear import protein for serine-arginine-rich (SR) proteins, is required for HIV-1 infection. In addition, cleavage and polyadenylation specificity factor 6 (CPSF6), which has a RS-domain that may be transported to the nucleus by TNPO3, binds to HIV-1 capsid (CA). Removal of the RS domain and/or nuclear localization signal causes CPSF6 to remain in the cytoplasm and restricts HIV-1 infection. HIV-1 with a mutation of asparagine 74 in helix 4 of HIV-1 CA to aspartic acid (N74D) does not bind to CPSF6 and does not require TNPO3 expression for infection. By tagging HIV-1 integrase (IN) with tagRFP in trans, we can label HIV-1 pre-integration complexes (PICs) and visualize them by live-cell, confocal, and super resolution microscopy. In addition, we have expressed the following proteins in HeLa cells by lentiviral transduction or transfection: full-length CPSF6 and truncated CPSF6 (CPSF6-358) tagged with eGFP, TNPO3 tagged with eGFP or tagBFP, and Nup153 tagged with iRFP670. eGFP-TNPO3 is mainly nuclear and co-localizes with iRFP670-Nup153 at the nuclear membrane, while CPSF6-eGFP is also mainly nuclear but does not co-localize with the other two proteins. HIV-1 PICs traffic efficiently to the nucleus within 1-2 hours after transfection when any of these proteins are expressed. However, when TNPO3 is knocked down in cells, N74D PICs and not WT PICs migrate toward the nucleus. In addition, CPSF6-eGFP expression is re-localized to the cytoplasm when TNPO3 is depleted. Similarly, CPSF6-358-eGFP expression in HeLa cells does not allow trafficking of WT PICs into the nucleus. Thus, TNPO3 and CPSF6 localization in the nucleus is required for efficient nuclear entry of WT HIV-1 PICs. Current studies are underway to visualize interactions of cores and/or PICs with CPSF6, TNPO3, and Nup153 under different conditions.

BACK

T50. Embryonic Lethality Due to Arrested Cardiac Development in Psip1/Hdgfrp2 Double-Deficient Mice

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Hepatoma-derived growth factor (HDGF) related protein 2 (HRP2) and lens epithelium-derived growth factor (LEDGF)/p75 are closely related members of the HRP2 protein family. LEDGF/p75 has been implicated in numerous human pathologies including cancer, autoimmunity, and infectious disease. Knockout of the *Psip1* gene, which encodes for LEDGF/p75 as well as the shorter LEDGF/p52 splice variant, was previously shown to cause perinatal lethality in mice. The function of HRP2 was by contrast largely unknown. To learn about the role of HRP2 in mouse development, we knocked out the *Hdgfrp2* gene, which encodes for HRP2, in both normal and *Psip1* knockout mice. *Hdgfrp2* knockout mice developed normally and were fertile. By contrast, the double deficient mice died at approximate embryonic day (E) 13.5. Histological examination revealed ventricular septal defect (VSD) associated with E14.5 double knockout embryos. To investigate the underlying molecular mechanism(s), RNA recovered from dissected embryo ventricular tissue was subjected to RNA-sequencing analysis on the Illumina platform. Bioinformatic analysis revealed several genes and biological pathways that were deregulated by the *Psip1* knockout and/or *Psip1*/*Hdgfrp2* double knockout. Expression levels of genes whose products are known to interact with LEDGF, including *Jpo2*, *Men1*, and *Mll* for the p75 isoform and *Sfrs1* for the p52 isoform, were not significantly altered by either *Psip1* knockout or the dual *Psip1*/*Hdgfrp2* knockout. The tumor growth factor (Tgf) beta-signaling pathway, which plays a role in cardiac morphogenesis during development, was by contrast significantly deregulated in the double knockout sample as compared to both control and *Psip1* knockout samples. We speculate that deregulated Tgf-beta signaling was a contributing factor to the VSD pathology and prenatal lethality of *Psip1*/*Hdgfrp2* double-deficient mice.

BACK

T51. CCL19 Mediates Efficient HIV-1 Infection of Naïve and Central Memory CD4$^+$ T Cells without Alteration of F-Actin Density or dNTP Concentration

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Background: Reservoirs of latent HIV-1 reside in both naïve (T_N) and resting central memory (T_{CM}) CD4$^+$ T cells. However, these cells are inherently resistant to HIV-1 infection *in vitro* due to multiple restrictions of virus replication, including filamentous (F)-actin density and SAMHD1 reduction of dNTP levels. Recent studies have demonstrated that the chemokine CCL19, which binds to the CCR7 receptor expressed on T_N and T_{CM} CD4$^+$ T cells, efficiently facilitates HIV-1 infection of these cells without inducing T cell activation. In this study, we asked whether CCL19 alleviated the HIV-1 restrictions resulting from F-actin density or diminished dNTP concentrations.

Methods: The relationship between F-actin density in resting CD4$^+$ T cells and HIV-1 infection was evaluated by using latrunculin A, which is a reversible inhibitor of actin assembly. F-actin density in T_N and T_{CM} cells cultured in the absence or presence of CCL19 was assessed by flow cytometry and confocal microscopy. To measure the effect of CCL19 on SAMHD1 activity, dNTP levels in the T_N and T_{CM} (± CCL19) cells, and in activated CD4$^+$ T cells, were also measured.

Results: Latrunculin A potently inhibited HIV-1 infection of CCL19-treated total resting CD4$^+$ T cells (IC_{50} ~ 80 nM). This viral inhibition strongly correlated with F-actin density. However, both flow cytometry and confocal microscopy analyses revealed that CCL19 did not impact F-actin density in either the T_N or T_{CM} CD4+ T cell subsets. Consistent with previous reports, the concentrations of all four dNTPs was extremely low in both T_N (range: < 0.004 to 0.015 pmol/10^6 cells) and T_{CM} (range: <0.004 to 0.077 pmol/10^6 cells) CD4$^+$ T cells versus activated CD4$^+$ T cells (range: 0.03 to 0.4 pmol/10^6 cells). CCL19 did not increase the dNTP concentrations in either the T_N or T_{CM} CD4$^+$ T cells, suggesting that SAMHD1 activity was not impacted.

Conclusions: CCL19 facilitates efficient HIV-1 infection of T_N and T_{CM} CD4$^+$ T cells without impacting either F-actin density or dNTP concentrations. Collectively, these data suggest that there are other major blocks to HIV-1 replication in resting CD4$^+$ T cells that still need to be identified.

T52. Microscopic Approach to Investigate Binding Partners of Vpu

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Viral protein u (Vpu) plays a central role in HIV-induced pathogenesis. It is the only transmembrane protein among the HIV accessory proteins and thus targets host membrane proteins. Vpu hijacks host SCF ubiquitin ligase complex (Cul1-Skp1-Rbx1-βTrCP) to ubiquitinate and downregulate the level of restriction factors including tetherin (BST-2) and CD4, thereby facilitating virus amplification and release. Here, we are able to use confocal microscopy to investigate the interaction between Vpu and its binding partners in mammalian cells. These include Skp1-βTrCP, CD4, BST-2 and SCAM3. In addition, the Ser-Ser double mutation in Vpu suggests its ability to abolish binding to Skp1-βTrCP complex but not to CD4, BST-2 and SCAM3. The fluorescence size exclusion chromatography (FSEC) also confirm that wild type Vpu forms a stable complex with Skp1 and βTrCP. These two methods provide a quick route in principle to screen and characterize all the potential Vpu-interacting proteins.

BACK

T53. HIV-1 Nef Hijacks Clathrin Coats by Stabilizing AP-1 Polygons

Qing-Tao Shen[1], Xuefeng Ren[1], Rui Zhang[1,2], Il-Hyung Lee[1] and James H. Hurley[1,2]

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HIV Nef downregulates cell surface proteins by hijacking the AP-1 and -2 clathrin adaptors. We show that HIV-1 Nef cooperates with the GTPase Arf1 to induce the oligomerization and activation of AP-1. We report the cryoEM structure of an HIV-1 Nef and Arf1-bound AP-1 assembly at 8 Å resolution. This cryoEM structure, when combined with the previously determined crystal structure of an AP-1:Arf1 dimer, led us to predict the formation a 40 nm-diameter AP-1 hexagons whose inner face contains binding sites for membrane lipids and proteins, whilst the outer face provides a platform for clathrin assembly. This prediction was validated by mutational analysis of Arf1- and Nef-promoted clathrin cage assembly in vitro. Arf1 and HIV-1 Nef thus play interconnected roles in allosteric activation, cargo recruitment, and coat assembly, revealing an unexpectedly intricate organization of the inner AP-1 layer of the clathrin coat.

BACK

T54. Probing Conformational States Of HIV-Host Complexes With Recombinant Fabs

Natalia Sevillano[1], Florencia La Greca[1], Hai Ta[1], Jennifer Binning[1], Xuefeng Ren[2], Bei Yang[2], James Hurley[2], John Gross[1] and Charles S. Craik[1].

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We have successfully generated recombinant Fabs that are useful for both functional and structural analysis of a wide range of targets, including proteases (PNAS 2013;110:93-98), receptors (Cancer Res. 2013; 73:2070-81) and ABC transporters (Nature. 2015;517:396-400). We have also used recombinant Fabs to analyze HIV-host complexes including 1) Vif-CBFb-ELOB-ELOC complex and 2) the clathrin adaptor complex AP-2 and Nef. A fully human naïve Fab (fragment antigen-binding) phage-display library with a diversity of 4.1 x 10^{10} was constructed using methods previously described (J Biol Chem. 2010;285:26878-88). We have optimized the protocols for phage display biopanning for fast verification of binders and initial characterization of the epitope by semi-quantitative and competitive ELISAs without the purification of the Fabs (Methods. 2011; 55:303-309).

Using these optimized phage-display methods we have identified six tight-binding Fabs against the VCBC complex (Vif-CBFb-ELOB-ELOC) with binding constants in the nanomolar range. All of the Fabs bind Vif complexes and one of them, Fab 3C9, inhibits ubiquitination of APOBEC3F *in vitro* but not A3G. Functional studies were then carried out to determine the effects of these reagents in cells. To avoid difficulties presented by a disulfide bond between the heavy and the light chain of Fabs, scFvs (single chain variable fragment), which contain only the variable fragments of an antibody fused in one chain, were constructed. The scFv of 3C9 was expressed in mammalian cells and shown to block the neutralization of A3C and A3F but not A3G. We have also made engineered versions of 3C9 that are less prone to ubiquitination (lys-free mutant) as well as a diabody of the scFV in an attempt to increase its effect *in vivo*.

For the Nef-AP2 project, we have identified three recombinant Fabs that bind specifically to AP2 muCTD (AP2 mu2 subunit C-terminal domain) and two Fabs that bind specifically to the AP-2 core. The specific antibodies for the AP2 muCTD promote the open conformation of AP2, and are being used for both functional and structural studies, including EM studies and for the co-crystallization of AP-2 and Nef with CD4. Other HIV-Host complexes are being pursued using this approach and will be discussed.

BACK

T55. The Genetic Interaction Landscape of HIV Infection in Human Cells

David E. Gordon, Ariane Watson, Assen Roguev, Raj Bhatnagar, David C. Crosby, Nevan J. Krogan

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Viruses such as HIV infect host cells, evade host immune defenses, and reshape the cellular environment by binding and co-opting specific pieces of cellular machinery known as host factors. A number of biochemical and genetic screens have identified host factors mediating HIV infection, however the functions of many remain poorly defined. There is a need for an improved genetic analysis of viral-interacting host factors, one which allows for deeper functional characterization to group functionally related genes and guide validation experiments. To this end we have developed a high-throughput genetic interaction (GI) mapping pipeline for interrogation of viral host factors in human cells, and used it to map the human host factors mediating HIV infection.

Quantitative mapping of GIs, by simultaneously perturbing gene pairs then measuring the resultant phenotypes, is a powerful tool for understanding complex biological phenomena permitting the unbiased characterization of gene function and the mapping of functional modules and pathways. Here we present a GI analysis pipeline to study the functions of HIV host factors through pairwise knockdown of 360 host genes (representing 129,600 combinations) followed by infection with HIV. Our work combines a luminescence-based viral infectivity assay to quantify HIV infection with a microscopy-based cellular analysis, allowing us to assemble GI maps of the host factor complexes and pathways mediating the early HIV lifecycle.

T56. Rapid Experimental SAD Phasing and Hot-Spot Identification with Halogenated Fragments

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Through X-ray crystallographic fragment screening we discovered 4-bromopyrazole to be a “magic bullet,” capable of binding at many of the fragment “hot spots” found in HIV-1 reverse transcriptase (RT). The binding locations can be in pockets “hidden” in the unliganded crystal form allowing for rapid identification of these sites for *in silico* screening. In addition to hot-spot identification, this ubiquitous yet specific binding provides an avenue for X-ray crystallographic phase determination, which can be a significant bottleneck in determining structures of novel proteins. The anomalous signal from 4-bromopyrazole or 4-iodopyrazole was sufficient to determine the structures for three proteins (HIV-1 RT, influenza A endonuclease, and proteinase K) by single wavelength anomalous dispersion (SAD) from single crystals. Both compounds are inexpensive, readily available, safe, and very soluble in DMSO or water allowing for efficient soaking into crystals.

BACK

T57. Incorporating Property-Based Volume Overlap with DOCK Descriptor Score

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Scoring functions are an essential component for accurate virtual screening and *de novo* design of lead-like compounds. We recently implemented a property-based volume overlap scoring function into the program DOCK, based on the algorithm reported by Sastry, et al. (*J. Chem. Inf. Model.* **2011**, *51*, 2455), to help identify lead compounds occupying similar space, with atoms of similar chemical properties, as a known reference ligand. The goal is to be able to include a volume overlap term along with other DOCK scoring functions, for example energies, pharmacohphore overlap, or molecular footprint similarity, among others. Under the umbrella of a function called “descriptor score”, linear combinations of different scoring functions allow DOCK users to screen for drug-like leads and design novel compounds with specific desired properties. Progress towards validation and application of volume overlap score alone and in combination with different scoring functions for virtual screening and *de novo* design will be discussed.

BACK

T58. Computational Design of Carbohydrate-Binding Proteins with Incorporation of Carbohydrate Flexibility and Mutation

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Lectins, carbohydrate-binding proteins capable of binding saccharides with high specificity, are of great interest due to their antiviral potency. The carbohydrates on the viral envelope play an essential role in shielding viruses such as human immunodeficiency virus (HIV) from the humoral immune response. Computational protein design programs provide us powerful tools to search on the sequence and structural spaces of protein, but they have limitation on the applicability in protein-carbohydrate systems. To apply computational protein design methods on protein-carbohydrate systems, one simplification is to make the carbohydrate target rigid and restrict variations to the protein moiety. However, it is unclear whether small differences in the input carbohydrate structure will expand to huge differences in the outcome of design calculations. Herein, we developed a general method for incorporating carbohydrate flexibility into a design algorithm for carbohydrate-binding proteins, which searches over the sequence and conformational spaces of both proteins and carbohydrates simultaneously. In addition, the method was extended to perform mutations on carbohydrates and thus it can serve as a potential tool to investigate the specificity of lectins toward a series of oligosaccharides with the same skeleton but different component units. The method was tested on Cyanovirin-N (CVN) and Microcystis viridis lectin (MVL) that bind high-mannose oligosaccharides and the core structure of N-linked oligosaccharides respectively. Detailed comparisons with the traditional rigid-carbohydrate method revealed that structural adjustments of carbohydrate in adaption to variations in the protein are essential for discovering low-energy configurations in the protein-carbohydrate systems.

T59. Estimation of the Binding Energies of Drug-like and Nondrug-like Molecules Docked in the Active Site of 1BIS.pdb

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After docking approximately 200 molecules in the active site of 1BIS.pdb the Cambridge Structural Database was used to identify the crystal structure that provided the best conformer representation. The binding energy was then computationally determined with an apparent division of the molecules into two groups — drug-like and nondrug-like. Data analysis was completed between the strand transfer and binding energy values.

BACK

T60. Protocol Development to Include Solvated Molecular Footprints in Lead Discovery

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In many disease-related systems, "bridging waters" (crystallographic water molecules at specific locations in the binding pocket) play a critical role in mediating hydrogen bonding between protein receptors and ligands. Bridging waters can be displaced by polar functionalities in rationally designed ligands which mimic the water-mediated interactions to improve binding affinity (*e.g.* cyclic urea inhibitors of HIV-1 protease). The interaction energy contribution of bridging water molecules can be observed by comparing molecular footprints (defined as per-residue energy decompositions) computed with and without waters present. This work is aimed at developing a semi-automated protocol which quantitatively identifies important bridging waters from other crystallographic waters in the binding pocket of a ligand-receptor system. A training set of systems reported with bridging waters and a test set of unexplored systems are currently under development. The goal is to use solvated molecular footprints containing bridging waters as a reference in large-scale virtual screening to identify new drug leads which mimic the interaction patterns made by the reference.

BACK

T61. Enrichment of Virtual Screening Results with Interaction Probe Analysis

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Techniques using known pharmacophores have been used to augment or evaluate docking methods and results. However, can a similar approach be used when no such information is available? A new post-docking analysis, currently called Interaction Probe Analysis (IPA), utilizes non-van der Waals protein-ligand interactions from a set of probe dockings to re-score docked compounds. Several sets of actives and decoys from the DUD-E Set were used to evaluate IPA, with sizes ranging from 3,000 to 36,000 compounds. The motivation for developing IPA is the eventual analysis of billions of dockings from the FightAIDS@Home Project on the World Community Grid, where one receptor will have over 5 million dockings. Accordingly, a BED-ROC metric with α = 500 is used to evaluate the top 0.2% percent of a docked library (AutoDock Vina) that is re-scored by IPA. IPA applied to HMG CoA Reductase, containing 9,000 actives and decoys, yielded BED-ROC(α = 500) = 0.89, resulting in 40 (80%) actives in the top 50 (0.55%) compounds. The corresponding enrichment factor is 27. Methodology and results are presented.

T62. A Genetic Algorithm for DOCK to Aid in *De Novo* Design

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Many computational *de novo* design methods combine molecular fragments in order to construct lead-like compounds to a known drug target. However, since fragments may have multiple attachment points, as the number of fragments increases, there can be an exponential growth of newly constructed ligands. In an attempt to prevent a combinatorial explosion, genetic algorithms (GAs) have emerged as a *de novo* design tool to drive compound generation towards a narrower chemical search space. GAs can also be used in conjunction with standard virtual screening by breeding top-ranked molecules in the context of the binding site to improve ligand-protein scores. The goal of this project is to implement a GA for the program DOCK that incorporates ligand binding interactions, as quantified by different scoring functions, in order to drive 3D assembly of novel small molecules towards a desired phase space. Progress towards this goal and current GA development will be presented.

BACK

F1. Structural Insights into an HIV Splicing Regulatory Complex

Christopher E. Morgan[1], Jennifer L. Meagher[2], Jeffrey D. Levengood[1], James Delproposto[2], Carrie Rollins[1], Jeanne A. Stuckey[2] and Blanton S. Tolbert[1]

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hnRNP A1 is a multifunctional RNA binding protein involved in a wide range of biological functions, including mechanisms of HIV splicing. While RNA stem loops represent a major class of hnRNP A1 targets, hnRNP A1-RNA interactions have been inferred primarily from the protein bound to ssDNA. We present the first high-resolution crystal structure (1.92 Å) of the two-RRM binding domain of hnRNP A1, UP1, bound to a 5'-AGU-3' trinucleotide, representing sequence elements of several HIV stem loops. The structure shows that hnRNP A1 interacts specifically with the AG dinucleotide sequence through a "nucleobase pocket" formed between RRM1 and the inter-RRM linker, where RRM2 does not come into contact with the RNA. Using Isothermal Titration Calorimetry and Molecular Dynamics simulations, we show that two conserved salt bridge interactions at the inter-RRM interface (R75:D155 and R88:D157) stabilize the linker in a geometry positioned to bind RNA with high affinity. We further investigated the structural basis of UP1 binding HIV $SL3^{ESS3}$ by determining a SAXS-scored structural model of the complex, showing that UP1 docks on the apical loop of $SL3^{ESS3}$ using RRM1 and the inter-RRM linker only. The biophysical aspects of the structural model were then examined by measuring kinetic binding parameters through Bio-Layer Interferometry, where mutations introduced within the apical loop reduce binding affinities by slowing down the rate of association. Collectively, the data presented here provide insights into how HIV uses RNA structures to utilize host splicing regulatory proteins.

F2. Combining PrimerID and Deep Sequencing to Quantify HIV-1 Splicing, Measure Mutation Rates, and Screen Mutations in Splice Control Elements

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HIV-1 RNA undergoes complex splicing to produce more than 40 different transcripts. Previous research on HIV-1 splicing used artificial constructs and single exon systems, and quantification was done by size and relative abundance of PCR products. It is now possible to quantify HIV-1 RNA splicing patterns in the context of viral infection using Illumina paired end deep sequencing and PrimerID. This quantitative tool can also be used to explore the role of splice regulatory sequences, using splicing patterns as a phenotype.

The first step in the assay is to infect cells with HIV-1 or transfect with an infectious molecular clone. Total cellular RNA is extracted and made into cDNA. A key part of this quantification assay is the primers used in this cDNA step. HIV splice variants can be divided into two size categories, the 1.8kb and 4kb classes. The 4 kb class includes a large exon which is spliced out of the 1.8 kb class. The 4kb class primer uses a sequence from this exon. The 1.8 kb class primer spans the splice junction. Thus each primer is specific to its size class.

Additionally, these two initial primers are tagged with PrimerIDs. The Primer ID is a sequence tag used to identify and quantify individual viral RNA templates. Reverse primers are synthesized with a random 13 nucleotide tail sequence. This creates 4^{13} combinations, over 67 million possible random sequences. Given a sufficient excess of random sequences relative to sample templates, any one viral RNA template will be tagged with a unique random sequence that is then incorporated into the cDNA and all PCR products made from that cDNA. After sequencing, all reads with a common PrimerID can be condensed into a single consensus read. This filters out mutations introduced by the PCR steps or sequencing errors and prevents quantification errors caused by PCR skewing.

This quantification protocol was used to observe APOBEC3G induced mutations to the region between splice acceptor 5 (A5) and splice donor 4 (D4). This region contains a regulatory element, ESE(GAR), thought to control splicing at both A5 and D4. Data from the splicing assay can be used to correlate a panel of mutations in the ESE(GAR) control region to both upstream and downstream splicing events.

BACK

F3. Solution Structure of the HIV Intronic Splicing Silencer (ISS)

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Alternative splicing is an essential step of the HIV life cycle, yet little is known about the structures that control splice site selection. Splicing from donor site D4 to acceptor site A7 removes the RRE to allow expression of late phase viral proteins. Given the importance of this splicing event to controlling viral replication, the activity of splice site A7 is tightly regulated by a complex network of an intronic splicing silencer (ISS), a bipartite splicing silencer (ESS3a/b) and an exonic splicing enhancer (ESE3). RNA chemo-enzymatic probing studies have shown the isolated splice site A7 locus folds into three RNA stem loops, where the regulatory elements localize to distinct apical loops. The host hnRNP A1 protein binds SL1(ISS) and SL3(ESS3) to effectively repress splice site A7, whereas the ASF protein counteracts hnRNP A1 by binding SL2(ESE3). To gain a better understanding of the RNA structures and protein interactions that regulate A7, we have solved the high-resolution structure of the ISS stem loop. ISS folds into a 53-nt long stem-loop RNA composed of several non-canonical structural features: a UG wobble tract, a stable 2X2 internal loop, a UU bulge and a 5-nt apical loop. As a step towards understanding how hnRNP A1 gets recruited to splice site A7, we have further characterized the interaction of its UP1 domain with ISS. UP1 binds to the apical loop with high affinity and specificity. Collectively, this data provides valuable insights into developing structure based mechanisms of HIV splicing.

F4. Targeting Dynamic Ensembles of Exon Splicing Silencer 3 Domain of HIV-1 Genomic RNA

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Non-coding RNAs in the HIV-1 genome play crucial roles in transcription regulation, alternative splicing and nuclear export, and may prove to be viable next generation therapeutic targets. We have previously developed methodology for determining dynamic ensembles of RNA employing a combination of advanced molecular dynamics simulations and experimental NMR parameters, such as chemical shift and residual dipolar couplings (RDCs) using HIV-1 Transactivation Response (TAR) domain as the model system (1). Subsequently these dynamic ensembles are then used to identify small molecules that bind and modulate their structure and activity by computational docking. Here, this methodology is applied to the Exon Splicing Silencer 3 (ESS3) domain of HIV-1, which binds the subunit UP1 of the host protein heterogeneous nuclear ribonucleoprotein (hnRNP) A1 to regulate HIV-1 splicing (2). We present preliminary ensembles for ESS3 at low (5.5) and high (7.5) pH and show that protonation of an A^+-C mispair minimally affects the dynamic properties. The ensemble suggests extensive and complex mobility within the apical loop, which requires additional NMR measurements to reliably determine the ensemble. To this end, we present proof-of-principle studies whereby ^{13}C and ^{1}H chemical shifts are also included in the ensemble determination. Preliminary results from computational and experimental screening targeting the ESS3 ensemble resulted in the identification of several tentative small molecule hits.

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2. Rollins C., Levengood J.D., Rife B.D., Salemi M., and Tolbert B.S. (2014). "Thermodynamic and Phylogenetic Insights into hnRNP A1 Recognition of the HIV-1 Exon Splicing Silencer 3 Element". *Biochemistry* **53**(13):2172-84. Levengood J.D., Tolbert M., Li M.L., and Tolbert B.S. (2013). "High-Affinity interactions of hnRNP A1 with conserved RNA structural elements is required for translation and replication of Enterovirus 71″. *RNA Biology* **10**(6):1-10.

F5. Examination of The Disordered C-Terminal Domain of a Host Regulator of HIV-1 Splicing

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Alternative splicing of the HIV-1 genome is necessary for translation of the complete viral proteome. Host proteins, such as hnRNP A1, are used to regulate splicing at the various donor and acceptor sites along the viral genome. The multi-faceted hnRNP A1 is composed of three domains, two structurally identical RRM domains that form the nucleic acid binding protein UP1, and a glycine rich C-terminal domain that is primarily involved in protein-protein interactions.

A crystal structure of UP1 was solved with a rAGU trinucleotide bound to the protein. This structure revealed that while only RRM1 binds the RNA, RRM2 is responsible for positioning several residues involved in the binding of the substrate. This mode of binding opens the possibility for allosteric regulation of hnRNP A1, presenting a need to study the structural properties of the C-terminus and its potential interactions with RRM2.

A preliminary view of the structure of full length hnRNP A1 has been obtained through SAXS-scored structural modeling. This model revealed the C-terminal domain to be a long, unstructured, disordered polypeptide chain extending away from the UP1 domain. In this model, the C-terminus appears to be independent of UP1 as there are no interactions between the two. The C-terminus is further being studied through the use of NMR with amino acid selective labeling. Mutations to mimic modification of the C-terminus are also being carried out. Finally, the binding of small molecules and peptides to the C-terminus is being examined through the use of both Isothermal Titration Calorimetry (ITC) and NMR.

BACK

F6. Mechanism of the DDX1-Assisted Assembly of Rev on the Rev Response Element

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The HIV-1 Rev (Regulator of Expression of Virion) protein activates nuclear export of unspliced and partially spliced viral mRNAs, which encode the viral genome and the genes encoding viral structural proteins. Rev interacts with a highly conserved region, the Rev Response Element (RRE), located within the viral mRNA. In order to activate nuclear export, Rev must bind to and oligomerize on the RRE. Using a novel single-molecule fluorescence assay, we previously showed that the assembly process begins with the binding of a single Rev monomer to stem-loop IIB of the RRE, whereupon additional Rev monomers are recruited to the RRE through a combination of RNA-protein and protein-protein interactions. We have also shown that the host protein DDX1, a member of the DEAD box family, promotes oligomerization of Rev on the RRE by accelerating the binding of the second and subsequent Rev monomers. Here, we use a variety of single-molecule spectroscopic methods to investigate the mechanism by which DDX1 achieves these effects. In principle, DDX1 could mediate Rev-RRE assembly by interacting with either the Rev protein or the RRE RNA, since both are known ligands for DDX1. Using a single-color assembly assay, we observed that DDX1 enhances oligomerization of a Rev mutant that is incapable of binding to DDX1, suggesting that DDX1 may act through the RRE RNA rather than Rev. To test the role of RNA binding more directly, we created a DDX1 mutant that is incapable of binding to the RRE and tested it's effect on Rev-RRE assembly using the same assay. Unlike wt DDX1, the DDX1 mutant did *not* promote Rev oligomerization on the RRE – the distribution of Rev-RRE complexes was similar to that of Rev and the RRE alone. Using two-color colocalization and FRET analysis, we monitored binding of Rev (labeled with Alexa 555) and wt DDX1 (enzymatically labeled with Alexa 647) to the same immobilized RRE molecule. Interestingly, after sorting the data into different groups, we found that Rev displayed enhanced oligomerization behavior, regardless of whether DDX1 was stably bound to the RRE or present in solution but not associated with the RRE during the observation period. Together, these results suggest a model in which DDX1 transiently interacts with the RRE, causing a persistent change in the secondary and/or tertiary structure of the RRE that facilitates subsequent Rev binding events. Supported by NIH P50 grant GM082545.

F7. A Mechanism for DDX1-Mediated Rev Binding to the RRE RNA: An RNA Structure-Driven Process

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HIV protein Rev (Regulator of Expression of Virion) association with and oligomerization on partially and fully unspliced HIV RNA transcripts is the first step in exporting late HIV RNA products from the nucleus. Rev specifically binds to stem II of the 351 nucleotide Rev Response Element (RRE) and subsequently oligomerizes utilizing a combination of its RNA and protein binding domains. This complex then acts as a substrate for native CRM-1 mediated nuclear export. Previously, we demonstrated that human DEAD-Box Protein 1 (DDX1) is required for the efficient export of unspliced HIV transcripts from the nucleus. This correlated *in vitro* with an increase in Rev oligomerization on the RRE in the presence of DDX1. However, the mechanism of this olgimerization enhancement remains unclear. Using native gel electrophoresis, single-molecule and bulk fluorescence and chemical-mediated structure probing techniques we have investigated the role of DDX1 in the Rev/RRE complex formation. We found that DDX1 and Rev independently induce similar secondary structure formation within the three-way junction of Stem II located on the RRE. While Rev does so by staying stably bound to the RRE element, DDX1 transiently interacts with the RNA and creates a "kinetically trapped" structure which Rev binds to with increased affinity. Further, DDX1 induces this reformed structure in the absence of exogenous ATP, albeit with lower efficiency than with ATP present. Unsurprisingly, mutants of DDX1 which affect its RNA binding ability also show a marked decrease in Stem II remodeling and subsequent Rev binding ability. However, mutations which affect direct interaction between DDX1 and Rev show no deleterious effects on either stem II remodeling or subsequent increased Rev association and oligomerization. In the absence of Rev, DDX1 causes an increase in intermolecular RRE/RRE oligomerization. However, in the presence of Rev, intermolecular RNA oligomerization is effectively inhibited. This data taken together indicate a model wherein DDX1 acts as an RNA chaperone, pre-folding the Stem II RNA into a conformation more advantageous for Rev binding than when unstructured. This initial increase in affinity translates to increased oligomerization due to the cooperative nature of Rev-Rev interaction on the RRE.

BACK

F8. Structural Investigation into the HIV RNA Nuclear Export Complex

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The human immunodeficiency virus (HIV) epidemic affects nearly 1% of the global population and additional drugs that target novel aspects of the virus life cycle may help in developing more effective treatments. The survival of HIV is contingent upon interactions between host and pathogen proteins and its ability to take advantage of the host's biological processes. HIV replication and particle assembly are dependent on the nuclear export of unspliced and singly spliced viral RNA transcripts; while the host, by contrast, retains unspliced RNA in the nucleus. In order to circumvent detainment of HIV transcripts two elements, an HIV regulatory protein (Rev) and a structured region of the viral RNA, assemble to form a ribonucleoprotein (RNP) particle. Rev is generated from the fully spliced viral transcript and oligomerizes on the Rev response element (RRE), a structured region within the intron of the unspliced and singly sliced viral transcript. The HIV RNP particle hijacks Crm1, a host nuclear export receptor that is regulated by the GTPase Ran, facilitating export of the viral RNA to the cytoplasm. Recent biochemical and structural experiments, including negative-stain EM, revealed a novel Crm1 dimer that is dependent on a species-specific interface that is critical to viral replication. Using single particle cryo-electron microscopy, our aim now is to determine a subnanometer resolution reconstruction of the Rev/RRE-Crm1/RanGTP complex. This structure in combination with biochemical assays will be used to probe and understand the mechanism of Rev-mediated HIV RNA export from nuclei. Specifically, we want to provide explanations for the role of the Crm1 dimer and Rev oligomer, decipher the orientation of Rev/RRE in relation to Crm1, and elucidate the details of the protein-protein and protein-RNA interfaces. Understanding the interfaces within viral/host complexes will aide in the discovery of novel therapeutics.

BACK

F9. Roles of RNA Helicases DDX1/DDX21 Nuclear Export and Stability of Unspliced HIV-1 mRNA

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Posttranscriptional regulation of HIV-1 gene expression is critical for virus replication. The HIV-1 transactivation protein, Rev, functions in the late stages of virus replication by promoting the nuclear export and translation of unspliced and partially spliced HIV-1 mRNAs that encode viral structural proteins. Rev binds and oligomerizes at the RRE (Rev responsive element) sequence which is present in the unspliced and partially spliced HIV-1 transcripts, and the Rev/RRE RNP complex is exported using Crm1 export receptor pathway. Previously, we identified the DEAD box RNA helicases DDX1 and DDX21 as Rev-interacting proteins and showed that they are important for HIV-1 replication and Rev function. We found that DDX1 or DDX21 silencing causes a decrease in p24 release from virus-infected cells and a reduced cytoplasmic accumulation of unspliced HIV-1 transcripts. However, it remains unclear how DDX1 and DDX21 influence the levels of unspliced HIV-1 transcripts. Based on studies of other members of DEAD box helicase family, we propose that DDX1/DDX21 influence the composition and structure of the viral mRNP complex, thereby promoting its stability and nuclear export. To test this hypothesis, we are studying the behavior of unspliced HIV-1 transcripts with DDX1 or DDX21 silencing. We generated Tet-On Advanced HEK293 cells with an integrated HIV-1 genome, in which transcription of native HIV-1 transcripts is activated by doxycycline. By utilizing dexamethasone-regulated activation of ectopic Rev and Tat, this system mimics the late stage of HIV-1 replication and allows analysis of the Rev-mediated export of unspliced HIV-1 RNA with physiological relevance. The roles of DDX1 and DDX21 in export and stability of the nuclear and cytoplasmic pools of HIV mRNAs are being analyzed by pulsed transcription approaches. Preliminary data suggests that the steady state levels of unspliced HIV-1 transcripts are decreased with DDX1 or DDX21 silencing, accompanied by a selective decrease in the level of the cytoplasmic pool of unspliced HIV-1 mRNA.

F10. Parallel High Throughput Experimental and Computational Screening Against a Flexible HIV RNA Target

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Non-coding RNAs are vital to the regulation of many cellular processes, making RNA an attractive target for next-generation therapeutics. HIV-1 transactivation response element (TAR) RNA is one example of a non-coding RNA vital for cellular regulation and disease progression. TAR binds to the viral trans-activator protein, Tat, resulting in transcriptional enhancement necessary for viral replication and pathogenesis. There has been significant effort towards identifying small molecules that can block the TAR-Tat interaction, however, the limited number of successes highlights the importance of developing new methods to target RNA.

Recently, we reported an approach for computationally screening RNA dynamic ensembles determined with the use of NMR spectroscopy and molecular dynamics (MD) simulations (Stelzer et al. 2011). Here, we describe parallel experimental and computational screening of >100,000 small molecules against TAR RNA to rigorously asses the performance of the ensemble-based docking approach. The experimental high throughout screen monitors the change in fluorescence upon displacement of a fluorescently labeled Tat peptide from TAR by the small molecule. We identify 11 hit compounds that bind TAR RNA with micro- to nanomolar affinity. A Receiver Operator Characteristic (ROC) analysis of the computational screening data gives an area under the curve (AUC) value of 0.88, which is on par with typical computational screens against well-folded proteins. Furthermore, the predicted ligand-bound structures are in good agreement with NMR chemical shift mapping data. Together this data highlight the power of parallel experimental-computational screens targeting dynamic RNA ensembles.

BACK

F11. Expansion of a Novel RNA-Binding Scaffold to Target HIV-1 *TAR* RNA

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The trans-activation response element (*TAR*) RNA is a well-studied and promising target in HIV-1 replication. Though several small molecule ligands have been developed, these ligands are often plagued by nonspecific interactions, inefficient cellular uptake, or toxicity. Recently, a molecular dynamics (MD)-generated and NMR-refined ensemble of 20 representative *TAR* conformations was used to virtually screen a large library of small molecules in order to identify new ligands. Experimental assays confirmed several hits as *TAR*-binding molecules, including dimethyl amiloride (DMA). Given the novelty of amiloride as an RNA-binding scaffold and its unique predicted binding mode, we have pursued the diversification and optimization of DMA through virtual and experimental screening. After successive rounds of docking and synthetic development, twenty-five analogs representing maximum possible variation in structure and selectivity of docking position were selected for further study. We have developed general synthetic routes for rapid, parallel synthesis of a series of diversified amiloride analogs by varying the substituents on two positions of the core pyrazine structure. These analogs were further evaluated using a fluorescent *TAR*-Tat displacement assay, which has revealed several derivatives with significantly greater activity when compared to DMA. Variations in the respective binding modes of these compounds to *TAR* were identified using the [^{13}C, ^{1}H] SOFAST-HMQC NMR technique.

F12. Novel *Cis*-Acting RNA Elements in the HIV-1 Genome

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The HIV-1 genome contains RNA sequences that are multi-functional in that they both encode proteins and perform superimposed functions during viral replication. Known elements include splice donors, acceptors, and branch points; *cis*-regulatory elements that enhance or silence nearby splice sites; the Rev-response element; the central polypurine tract; the central termination sequence; GagPol ribosomal frameshift regulatory elements; and part of the packaging signal.

To determine whether there are additional critical, but as yet undiscovered *cis*-acting elements in the HIV-1 genome, we undertook a global silent mutagenesis strategy. A mutant, full-length HIV-1 sequence was designed that contained a maximum number of synonymous mutations in all open reading frames. However, all previously identified RNA elements were kept intact and no new AG or GT dinucleotides were added to avoid creating new splice donors and acceptors. The silent mutations were divided into 150-500 nucleotide blocks, which were synthesized and cloned to generate sixteen different mutant proviruses. The sixteen different mutant viruses can be divided into three phenotypic groups: (1) those whose replication did not appear to be affected by the mutations, (2) those that exhibit clear splicing and replication defects, and (3) those that do not display obvious splicing defects, yet have clear replication defects. The phenotypic groups map to distinct regions of the genome. Viruses with silent mutations in Pol were fully replication competent. Conversely viruses with silent mutations in the central region of the genome (Vif, Vpr, Tat, Rev, Vpu) where many splice sites reside, exhibited both splicing and replication defects. Silent mutations in the Gag and Env open reading frame affected replication without inducing major perturbations in splicing. Single point mutations that arose following passage of viruses in the group with clear splicing defects were frequently able to restore replication competence, while the replication defective group with no apparent splicing defects had more complex requirements for restoring replication competence. Our data suggests that RNA elements in various regions of the HIV-1 genome might have multiple roles in viral replication that have not been previously identified.

F13. Data Mining for RNA Chemical Shift Predictions

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The elucidation of RNA structures has proved problematic in all structure solving techniques. Specifically with NMR, obstacles arise with larger RNAs due to weak inter-residue scalar couplings and limited chemical shift dispersion. To help guide RNA peak assignments of larger RNAs, a computer program (RNAShifts) was developed. Based on 1H NMR chemical shift analysis of RNA structure depositions from the Biological Magnetic Resonance Data Bank (BMRB), RNAShifts enables convenient prediction of experimental RNA 1H NMR assignments. Here we show how we have improved this analysis and prediction model. The model has been expanded to include carbon nuclei as well as hydrogen nuclei. We have automated the process in two ways. First, we can automatically retrieve NMR assignments from the BMRB and structural data from the PDB. Second, we can automatically analyze the data to derive secondary structure attributes. This automated process has greatly increased the data from our previous manually managed database. The focus of our analysis has now increased to the central base pair within a stretch of five consecutive nucleotides. This includes secondary structure attributes such as protein interactions loops, multiplets, and pseudoknots. We are now using Support Vector Regression, which allows these attributes to contribute in non-linear modes. These improvements have allowed us to increase the amount of data and thereby increase the number of nucleotide attribute combinations the program uses for prediction of new datasets.

BACK

F14. Role of HIV-1 Genomic RNA Structure in Human Lysyl-tRNA Synthetase Recruitment

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Human lysyl-tRNA synthetase (LysRS) and $tRNA^{Lys}$ isoacceptors are specifically recruited into HIV-1 particles where $tRNA^{Lys3}$ serves as the primer for reverse transcription. The mechanism by which $tRNA^{Lys3}$ is recruited into HIV-1 is incompletely understood. We have recently shown that LysRS binds to a U-rich loop in the 5´UTR region of the viral RNA genome immediately upstream of the primer-binding site (PBS). This interaction competitively displaces $tRNA^{Lys3}$ from LysRS, providing a plausible mechanism for primer release from LysRS and targeting to the PBS (1). This U-rich loop is a tRNA-like element (TLE) that mimics the $tRNA^{Lys}$ anticodon loop, a critical recognition element for LysRS. We recently reported the small-angle X-ray scattering (SAXS) analysis of a 105-nt RNA (PBS_{105}) containing the PBS and TLE of the NL4-3 5´UTR, both in the absence and presence of an annealed $antiPBS_{18}$ DNA oligonucleotide, revealing that the region further mimics tRNA by adopting a similar overall shape (2). However, binding assays show that the most high-affinity binding of LysRS requires SL1 and SL2 of the Psi packaging domain. New RNase protection assays indicate that LysRS elicits a dose-dependent protection of residues within the Psi domain, suggesting direct interactions.

While NL4-3 (representative of clade B) is one of the most studied HIV-1 isolates, another frequently examined isolate is MAL (representative of clade A in its 5´UTR). Despite the differences in their experimentally-determined secondary structures (3), we show that the affinity of LysRS for the NL4-3 and MAL PBS/TLE domains is similar, and SAXS analysis reveals that the two viral isolates share a strikingly similar overall shape. As for NL4-3, extending the MAL PBS/TLE RNA to include Psi increases the affinity of LysRS for this construct. Investigation of larger RNA constructs encompassing the PBS/TLE and Psi domains of the 5´UTR of each isolate using SAXS is underway in order to determine if overall structural homology may play a role in high-affinity recognition by LysRS.

1. Jones CP et al. *RNA* 19(2): 219-29 (2013)
2. Jones CP et al. *Proc Natl Acad Sci USA* 111(9): 3395-400 (2014)
3. Goldschmidt V et al. *J Biol Chem* 279(34): 35923-31 (2004)

BACK

F15. Modifications of Nucleic Acid Bases on tRNA^{Lys3} Affect Interactions with the Matrix Domain of the HIV-1 Gag Polyprotein

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HIV-1 packages host tRNA^{Lys3} into new viral particles. Upon infection, the tRNA is used as a primer by reverse transcriptase to transcribe the RNA genome into DNA. CLIP studies have found binding between the matrix domain of the HIV-1 Gag polyprotein and the tRNA primer, suggesting a mechanism for packaging of the primer. To investigate the role of nucleic base modifications on the interactions between matrix and tRNA^{Lys3}, we used EMSA on tRNA that contained both modified and unmodified bases. tRNA^{Lys} purified from *E. coli* cells, and thus containing the modified bases, showed binding, but assays using synthesized tRNA without modified bases do not. These results suggest that the modifications may play a role in the specificity of the binding between matrix and the tRNA primer.

BACK

F16. RNA Helicase A is Co-Packaged with HIV-1 RNA via Direct Interactions with the 5'-Leader RNA

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RNA Helicase A (RHA) is a cellular factor involved in many steps of the HIV-1 life cycle. An average of two RHA molecules are packaged per virion, and the RHA deficient virions exhibited impaired infectivity. We have obtained evidence showing that co-packaging of RHA is facilitated by the 5'-Leader of the HIV-1 RNA genome. Recent NMR studies of the HIV-1 5´-Leader RNA have suggested that the dimeric conformation exposes a large number of high-affinity nucleocapsid (NC) protein binding sites and thus promotes viral genome packaging. Therefore we hypothesized that the interaction between RHA and the dimeric 5´-Leader directs co-packaging of RHA into virions. To identify which residues are critical for RHA co-packaging, we generated dimeric 5´-Leader RNAs containing deletions and mutations without disturbing the overall structure, and tested their RHA binding properties. Cell-based packaging experiments were conducted with vector RNAs harboring the same mutations as used in the in vitro studies. We have identified the critical RNA element within the dimeric 5´-Leader to facilitate RHA co-packaging. Consistent with its previous reported roles in viral infectivity, our results provide structural insights into the RHA co-packaging mechanism.

BACK

F17. Co-Transcriptional Packaging of Retroviral Genomic RNA by the Gag Polyprotein

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Retroviruses use their unspliced RNA as both a template for protein synthesis (mRNA) and as the viral genome (gRNA). The retroviral Gag polyprotein selects gRNA for encapsidation into new virus particles through a high-affinity interaction with the psi packaging sequence in the 5' leader sequence. However, the mechanism by which Gag finds the genomic RNA amidst the sea of cellular RNAs in the infected cell is not well understood. It was originally thought that Gag functioned only within the cytoplasm. However, recent evidence suggests that a subpopulation of the Gag proteins of numerous retroviruses, including human immunodeficiency virus (HIV), Rous sarcoma virus (RSV), feline immunodeficiency virus, murine leukemia virus, mouse mammary tumor virus, and Mason-Pfizer monkey virus localize to the nucleus.

Our genetic and biochemical data indicate that RSV Gag nuclear trafficking is required for efficient gRNA packaging. Confocal microscopy revealed that RSV Gag is associated with nascent viral RNAs at sites of active transcription. Subcellular fractionation experiments demonstrated that Gag segregates with euchromatin, the transcriptionally active fraction. Using affinity purification followed by mass spec, we identified a set of host binding partners of Gag that regulate transcription, chromatin-remodeling, and splicing. We propose that Gag may bind to euchromatin-associated factors as a way to find transcription sites. Our data support a model of co-transcriptional packaging of RSV gRNA by nuclear Gag.

To determine whether the HIV-1 Gag protein has similar properties, we examined cells that express a dox-inducible, HIV genome that produces Gag-GFP in place of Gag-Pol. To visualize HIV-1 RNA, we used a FISH probe that specifically labels unspliced HIV RNA. With confocal microscopy, we observed small foci of Gag within the nucleus, where a subpopulation was colocalized with the unspliced viral RNA at sites of transcription. Moreover, HIV-1 Gag is also found in the euchromatin fraction. Together, these results suggest that nuclear trafficking of retroviral Gag proteins and their association with unspliced viral RNA at sites of transcription may be a conserved mechanism for selection of gRNA as a way to bind the unspliced viral RNA before it becomes spliced or exported into the cytoplasm as mRNA.

F18. How Does HIV-1 mRNA Initiate Viral Protein Synthesis Independently of eIF4F?

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Cap-dependent initiation is a highly choreographed process dependent upon cap-associated protein factors, the predominant eIF4F or the non-canonical CBP80/20, to recruit the ribosome and initiate translation. We hypothesized that the association of HIV-1 mRNA with these cap-associated factors was necessary to human retrovirus translation. Using co-immunopreciptiation analyses and polysome profiling, we identified HIV-1 generates an alternative cap-dependent translation initiation complex that works independently of eIF4F. The HIV-1 Translation mRNP (T-RNP) is composed of DXH9/RNA helicase A (RHA) and CBP80/20 cap-binding complex. Our studies characterized RHA selectively recognizes and binds a distinct RNA element, termed the posttranscriptional control element (PCE), in the 5' leader of HIV-1 mRNAs. This association stimulates ribosome loading and viral protein synthesis during downregulation of eIF4F by viral or exogenous stimuli. The RHA-CBP80/20 translation initiation mechanism used by HIV-1 was identified for a cohort of cellular mRNAs that are translated during cellular stress.

F19. Structural Isoforms of HIV-1 5' Untranslated Region Expose Distinct Protein Binding Sites that Promote Utilization as mRNA or Genomic RNA

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Structural studies have shown the 5'UTR of HIV-1 adopts mutually exclusive RNA conformations in solution that expose the AUG start codon or the GCGC dimerization initiation sequence (DIS). Biochemically, these RNA conformers are observed as monomeric or dimeric molecules, respectively. Biophysical studies have shown the dimer conformation exposes a large number of high-affinity binding sites of nucleocapsid (NC) protein, which are important to promote dimer RNA packaging. We tested the corollary hypothesis that the monomer conformation exposes protein binding sites important to promote mRNA translation. We generated monomeric 5´-UTR structural variants by nt replacements that favor AUG-exposed and measured its efficiency to template viral protein synthesis. Translation assays demonstrated the monomeric 5' UTR is more efficiently translated than either wild-type or dimeric HIV RNA. ITC demonstrated monomeric 5' UTR exposes several binding sites for RNA helicase A, whereas dimeric 5' UTR exposes a single binding site. Ribosome profile analysis and co-precipitation experiments demonstrated RHA facilitates cap-dependent translation initiation and polypeptide synthesis. In conclusion, monomer and dimer HIV 5' UTR structures engage mutually exclusive RNA-protein complexes that drive its utilization as mRNA template for translation or virion precursor RNA that becomes packaged in progeny virions.

BACK

F20. Understanding HIV-1 Packaging Signal by Single-Molecule Spectroscopy

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Selective packaging of the genomic RNA (gRNA) is still one of the least understood steps in the HIV-1 life cycle. On the one hand, deletion of the packaging signal (ψ) for HIV-1 shows ~ 50-fold reduction in the packaging of gRNA. On the other hand, a series of *in vitro* studies have shown that recombinant Gag can exhibit a nanomolar affinity not only for ψ but also for other RNA sequences. The fact that Gag binds to and assembles efficiently on other, cellular RNAs suggests that differences in affinity may not be sufficient to explain selective packaging of psi-containing RNA *in vivo*.

It seems likely that binding of retroviral Gag to nucleic acids is cooperative, but this has never been demonstrated. It is possible that cooperativity contributes to selective packaging; for example, binding of Gag to ψ-containing RNAs might exhibit a higher degree of cooperativity than for other RNAs.

Characterization of the binding of Gag to RNAs is complicated by the fact that under most experimental conditions, mixing Gag and RNA leads to virus-like particle assembly. We are approaching this challenge by using a *single-molecule* technique (Fluorescence Correlation Spectroscopy [FCS]), which allows the simultaneously determination of the diffusion coefficient (D) of fluorescently labeled Gag, RNA and Gag/RNA complexes. The power of this technique lies in the fact that FCS is performed under very dilute conditions (~ 1-10 molecules within the confocal volume [~0.25 fL]).

We have labeled several 190-nt RNAs (HIV-1 ψ, HIV-1 Gag-Pol frame shift [GRPE] and MoMLV ψ) with Cy5 at their 3' ends, and recombinant Dp6 HIV-1 Gag with AlexaFluor 488. We have determined that RNA collapses upon Gag binding and that under our conditions only a small number of Gag molecules (1-3) binds to the RNAs. By measuring the D of the RNAs and Gag/RNA complexes, as well as the degree of quenching of Cy5 (due to Gag binding), we have determined the K_Ds for HIV-1 ψ (in both monomeric and dimeric form), GRPE and MoMLV ψ. We have determined that amongst the tested RNAs the dimeric form of HIV-1 ψ has the lowest K_D. However, this difference in affinities does not seem sufficient to explain packaging selectivity. Furthermore, selective binding to ψ is only apparent in the presence of a large excess of tRNA. The data strongly support a cooperative model for binding of Gag to ψ. Ongoing experiments are characterizing the role of the MA domain in specific and nonspecific interactions with RNAs.

BACK

F21. Characterization of HIV-1 5'-Leader Dynamics using NMR Relaxation Measurements

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The 5'-leader is the most conserved region of the HIV-1 genome and is implicated in a number of functions necessary for retroviral replication including translation, genome dimerization and packaging. Understanding the mechanisms underlying these wide-ranging functions and their regulation requires detailed knowledge of its structure and dynamics.

NMR relaxation measurements offer uniquely powerful insights into site-specific motions on a range of timescales, however methods suitable for large RNA molecules are not yet established. We have therefore been developing and optimizing these methods, and will present initial results of these studies with the dimeric HIV-1 5'-leader.

BACK

F22. Characterization of Monomer/Dimer Equilibrium of the HIV-1 5'-Leader with Variable +1 mRNA Start Sites and Presence of the 5'-Methylguanosine Cap

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Despite the number of successful antiretroviral therapies implemented to combat the AIDS pandemic, HIV continues to be a global threat. The most highly conserved region of the HIV-1 genome is the 5'-Leader (5'-L), which consists of the first 356 nucleotides of the genome. The first 335 nucleotides are not translated into protein, instead the nucleotides of the 5'-L fold into distinct three dimensional structures which control a number of important steps in the HIV life cycle. The structure of the 5’-L has been studied extensively through secondary structure predictions, chemical probing, and NMR studies. As a retrovirus, HIV utilizes host cellular machinery to transcribe its RNA, resulting in fully processed transcripts that contain both a 5’-7-methylguaonsine cap and a PolyA tail. Despite the fact that the 5'-L is capped *in vivo*, most *in vitro* studies are performed on uncapped RNA. For these studies, *in vitro*, T7 transcribed HIV-1 5'-L was enzymatically capped utilizing a *Vaccinia* virus capping system in order to determine if the native, capped 5'-L shared the same dimerization properties as the uncapped 5'-L. Unexpectedly the presence of the 5'-methylguanosine cap stabilized the monomeric conformation of the 5'-L. Further investigations revealed that the presence of an additional 5'-guanosine also stabilized the monomeric conformation. A detailed literature review revealed inconsistencies in the reported 5'-start site of the HIV-1 genomic RNA. There are three guanosine residues in an appropriate position to be the +1 mRNA start site, and different groups report different residues as the +1 mRNA start site. This study characterizes the relative monomer/dimer equilibria of the HIV-1 5'-L beginning at any of the three possible start sites, with and without the presence of the 5'-cap. The differences in the monomer/dimer equilibria of these constructs reflects the importance of determining the correct 5'-start site of the genomic RNA. It is possible that all three potential start sites are utilized in varying percentages at different times during HIV infection, and this should be investigated as well.

BACK

F23. Direct Probing of the Dimer Interface in the 688-Nucleotide HIV-1 Leader RNA

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During assembly of the human immunodeficiency virus (HIV-1), two copies of the unspliced viral RNA genome are efficiently incorporated into virion. Previous studies have shown that the RNA in virions exists as non-covalently linked dimers, and dimerization is primarily promoted by the palindromic sequence GCGCGC located in the dimerization initiation site (DIS) within the 5'-untranslated region (5'-UTR). However, it is unclear whether the RNA exists as a kissing dimer (formed by kissing-loop interaction between two DIS stem loops), or an extended dimer (in which DIS unfolds and forms intermolecular base pairs with a second molecule). Traditional Nuclear Magnetic Resonance (NMR) techniques would return identical chemical shifts from each possible structure due to equivalent base pairing. Additionally, the molecule size greatly limits the application of NMR in RNA studies, due to severe signal overlap and line broadening associated with the increase in RNA size. We have developed a novel ^{2}H-edited NMR approach in which two identical RNA samples with different ^{2}H labeling combinations were made so that when mixed, if the two samples dimerized via formation of an extended dimer, signature Nuclear Overhauser Effects (NOEs) would be observed. Intermolecular interactions were confirmed by detection of cross-strand NOEs between A268-H2 and G251-H1', and also between A269-H2 and U250-H1' within the DIS, suggesting that the 5'-UTR forms an extended dimer. This method allows for the direct determination of the inter- and intramolecular base pairings within the HIV-1 5'-UTR dimer (688nt, 220kDa), and offers insight for determining the mechanism and structure of dimer formation.

BACK

F24. Characterizing RNA Dimerization Mechanisms of the Genomic 5´-Leader in Human and Simian Immunodeficiency Viruses

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Previous studies suggest that HIV-1 5´-leader (5´-L) RNA genome exists as an equilibrium mixture of monomeric and dimeric species, which is regulated by a molecular structural switch. In monomeric form, the dimer-promoting GC-rich loop of the dimer initiation site (DIS) hairpin is sequestered by base pairing with the unique-5´ region (U5). In dimeric form, a distal stem-loop region containing gag start codon (AUG) interacts with the U5 exposing the DIS. Consequently, the U5:AUG interaction occurs and promotes dimerization of HIV-1 5´-L RNA.

We asked a question whether this HIV-1 5´-L dimerization mechanism is ubiquitous among its related families of retroviruses. Sequence alignments, gel electrophoresis, ITC, and NMR methods are employed in this study to compare RNA dimerization behaviors among HIV-1_{NL4-3}, $SIV_{cpzTAN1}$, SIV_{cpzUS} ,and HIV-2_{ROD} strains. Interestingly, SIV_{cpz} and HIV-2 dimeric species appear to be labile and only detectable under TB running buffer with additional Mg^{2+} for the native gel electrophoresis method, whereas HIV-1 5´-L forms non-labile dimer detectable under TB running buffer in the absence of Mg^{2+}. Despite the difference in dimeric RNA lability, our findings strongly indicate that the 5´-L of HIV-1, SIVcpz, and HIV-2 utilize a similar RNA structural switch mechanism. This suggests the conserved 5´-L dimerization mechanism as a potential new target for anti-viral drug.

BACK

F25. Characterization of HIV-1 Gag-gRNA Interactions

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Despite a vast excess of cellular RNA, HIV-1 specifically packages a single copy of full-length, dimeric, genomic RNA (gRNA) into virions. The so-called psi stem-loop elements within the gRNA 5´-untranslated region (5´UTR) are critical for specific Gag-gRNA interactions. However, under physiological conditions, *in vitro* binding assays are not consistent with a significantly higher Gag binding affinity for psi versus non-psi RNA, and in the cytosol, Gag favors binding to the viral genome over cellular mRNAs by only a few fold, a level of discrimination that is unlikely to be sufficient to account for the high selectivity of gRNA packaging. We recently developed a fluorescence anisotropy salt-titration approach to characterize Gag-RNA binding in a quantitative manner (1,2). This approach allows determination of two binding parameters: $K_{d(1M)}$, which describes the non-electrostatic strength of binding, and Z_{eff}, the effective positive charge of the protein-nucleic acid interaction. Compared to other RNAs tested, Gag binds to psi RNA with a dramatically reduced $K_{d(1M)}$ and lower Z_{eff}. Mutations involving the zinc finger motifs significantly reduce the non-electrostatic component of binding and lead to an increase in Z_{eff}. A Gag variant lacking MA bound both RNAs with more similar Z_{eff} and $K_{d(1M)}$ values, indicating that the MA domain is primarily responsible for the increased electrostatic interactions observed in Gag/non-psi RNA binding and that the presence of MA increases Gag's ability to discriminate psi from non-psi RNA. Mutation of the RNA reveals that NC interaction sites within psi play non-equivalent roles in inducing the specific Gag binding mode. In addition, while dimerization does not affect Gag binding to a smaller ~100-nt psi RNA, dimerization plays a larger role in the case of the entire 5´-UTR.

The salt titration results predict that RNA binding modulates Gag conformation. We have initiated small-angle X-ray scattering (SAXS), electron microscopy (EM) and hydrogen-deuterium exchange mass spectrometry (HDX-MS) studies to test this hypothesis. SAXS analysis of monomeric WM-Gag∆p6 revealed multiple acceptable models with varying distances between CA and MA. Preliminary negative stain EM images of WM-Gag∆p6 alone suggest that this is a viable approach to visualize distinct Gag conformations. HDX-MS results show that addition of psi RNA leads to weak-to-modest protection from deuterium uptake in the trimer interface of MA, the CA CTD dimer interface, and SP1, while slightly more protection was observed in SP2.

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F26. Structural and Functional Insights into the Binding of Human TSG101 with a Small Molecule HIV-1 Budding Inhibitor

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The human ESCRT machinery is required for membrane remodeling events including multivesicular body biogenesis, cellular abscission, and viral budding. Specifically, the PTAP motif of retroviral Gag proteins, including HIV-1 Gag, targets the ESCRT-I complex through a direct interaction with TSG101 (tumor susceptibility gene 101). Through screening for disruption of Gag-Tsg101 interaction, we found a small molecule inhibitor of HIV budding, which we expected to bind to the PTAP recognition site of TSG101. Interestingly, although this inhibitor was found to bind to the N-terminal UEV domain TSG101, the interaction was largely outside of the PTAP binding site. The interaction was covalent and closer to the region in TSG101 that binds ubiquitin.

Using solution nuclear magnetic resonance (NMR), we investigated the structure of this unusual covalent complex of TSG101 with the small molecule inhibitor. Solving the structure of the protein was straightforward due to the small size (17 kDa) of the N-terminus of TSG101 and the previously solved structure in the free form (1). However, in order to determine the correct orientation of the ligand in the binding site, we needed data on the ligand itself. For non-covalently bound ligands, we can exploit the difference in molecular size between ligand and protein using saturation transfer difference or WATERLOGSY experiments. In our case, the covalent interaction precluded these types of experiments. Instead, we used $^{12}C/^{13}C$-filtered NOESY experiments to differentiate signals from the ligand (^{12}C) and protein (^{13}C) to aid in structure elucidation. We also investigated the effects of this small molecule on ubiquitin and PTAP binding to TSG101.

This structure of TSG101 in complex with a small molecule inhibitor of HIV budding can be used as a starting point for the design of novel compounds targeted at the Gag-Tsg101 interaction and the viral budding process. In addition, functional aspects of PTAP and ubiquitin binding to TSG101 can be investigated to provide additional insight into the role of the protein in ESCRT-mediated events.

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F27. Angiomotin Functions in HIV-1 Assembly and Budding

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Many retroviral Gag proteins contain PPXY late assembly domain motifs that recruit proteins of the NEDD4 E3 ubiquitin ligase family to facilitate virus release. To exit infected cells, HIV-1 usurps the cellular ESCRT pathway using PTAP and YPXL late domain motifs within $p6^{Gag}$ to bind and recruit TSG101/ESCRT-I and ALIX, respectively. Although HIV-1 lacks a PPxY motif and has not been shown to bind directly to any NEDD4 ligase, overexpression of NEDD4L can stimulate HIV-1 release, suggesting that NEDD4L may function through an adaptor protein. We have now shown that the cellular protein Angiomotin (AMOT) can bind both NEDD4L and HIV-1 Gag and act as an adapter to stimulate budding through NEDD4L. HIV-1 release and infectivity are stimulated by AMOT overexpression and inhibited by AMOT depletion. Electron microscopic analyses revealed that in the absence of AMOT, assembling Gag molecules fail to form a fully spherical enveloped particle[1]. To understand this process in greater detail, we have investigated the biochemistry and functional importance the of the three different PPxY motifs in the N-terminal region of AMOT p130, and the four WW domains in the central region of NEDD4L. AMOT PPxY-mutants that cannot bind NEDD4L also fail to promote virus release, and mutations in individual WW domains support HIV-1 budding to different degrees. *In vitro* binding affinities between isolated AMOT WW-domains and PPxY-motifs generally correlate with functional importance, with the highest affinity interactions also the most important for virus budding. Our experiments indicate that AMOT, and other motin family members, must function together with NEDD4L to help complete immature virion assembly prior to ESCRT-mediated virus budding.

1. Mercenne G, Alam SL, Arii J, Lalonde MS, Sundquist WI. Angiomotin functions in HIV-1 assembly and budding. Elife. 2015 Jan 29;4.

F28. Identification of a Novel Structural Element in Gag Important for the Assembly, Release and Maturation of HIV-1 Particles

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During the assembly process, Gag, the main viral structural polyprotein of HIV-1, forms a hexameric protein lattice at the plasma membrane. Interactions between capsid (CA) domains of Gag are indispensable for formation of the immature Gag lattice. CA is primarily alpha-helical, and is comprised of an N-terminal domain (CA-NTD) and C-terminal domain (CA-CTD), joined by a flexible linker.

We performed alanine-scanning mutagenesis to investigate the function of a highly conserved Pro-Pro-Ile-Pro motif (CA residues 122-125) in the loop connecting helices 6 and 7 (the H6-H7 loop) of the CA-NTD. We observed that mutations P122A and I124A significantly impaired virus release, particle infectivity, and replication in a T-cell line. In contrast, mutations P123A and P125A were reasonably well tolerated. In addition, we demonstrated that P122A and I124A mutant virions lack the characteristic cone-shaped central CA core, and also have an abnormal immature Gag lattice in protease-deficient particles. Through prolonged viral passage in multiple T-cell lines, we identified a set of compensatory mutations located at distal sites in the CA-NTD that rescue the functional defects caused by the P122A and I124A substitutions. Two mutations, V11I and T58A, when present together, rescue the defects imposed by both P122A and I124A such that V11I/T58A/P122A and V11I/T58A/I124A triple mutants are assembly competent, infectious, and replicate with near-WT kinetics. The mechanism by which mutations at distal sites in CA are able to rescue the defects imposed by H6-H7 loop mutations is currently under investigation.

According to a recently published structure of the CA domain in the immature Gag lattice (Schur et al., Nature 2015), the H6-H7 loop is located at the interface formed by three CA monomers from adjacent hexamers. Together with our data, this suggests that the H6-7 loop of the HIV-1 CA domain is a new structural element necessary for interhexamer contacts in the immature Gag lattice.

F29. The Matrix Trimer Is an Essential Structural Component of the Infectious HIV-1 Particle

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The matrix (MA) domain of HIV Gag has important functions in directing the trafficking of Gag to sites of assembly, and mediating the incorporation of the envelope glycoprotein (Env) into assembling particles. HIV-1 and SIV MA proteins have been proposed to oligomerize as trimers, based on studies using recombinant MA in either 3D-crystallography or 2D protein lattices arranged on a lipid membrane. However, the presence of MA trimers in infectious HIV-1 virions has not been documented. To this end, we developed a biochemical cross-linking strategy to reveal MA trimers in mature and immature virions of replication-competent HIV-1. We further exploited this cross-linking strategy to address the nature of the MA trimer interface, identifying several key residues that are required for HIV-1 MA trimer stability. By mutating these residues, we were able to demonstrate a correlation between loss of MA trimerization, and loss of Env incorporation and viral replication. To address the role of MA trimerization in the viral replication cycle, we rescued virion infectivity through two distinct approaches. First, we demonstrated that short-tailed envelope proteins can be incorporated into MA trimer-defective particles and confer infectivity, and that MA trimer-defective mutants bearing a truncated Env cytoplasmic tail (CT) were replication competent in MT4 cells, a T-cell line permissive for the replication of CT-truncated HIV-1. Second, we passaged MA trimer-defective mutants in T cells and were able to identify second-site mutations that were proximal to the MA trimerization interface; these second-site mutations restored Env incorporation, particle infectivity and viral replication. By using these complementary loss-of-function and rescue approaches, we demonstrate that MA trimerization is primarily important for the incorporation of Env into particles. Combining our data with the available MA structures, we propose a model whereby MA trimerization is required to form a lattice capable of accommodating the long CT of HIV-1 Env. In the absence of MA trimerization, Env would then be sterically excluded from the assembling particle. These data reveal that the MA trimer represents an essential structure in the HIV-1 particle, and suggest that the MA trimer interface may represent a novel drug target.

BACK

F30. *In vivo SELEX* Reveals Novel Protein Determinants for Murine Leukemia Virus (MLV) Envelope Glycoprotein Functionality

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Enveloped viruses utilize membrane-spanning cell surface glycoproteins to mediate fusion between viral and host cell membranes. Viral glycoproteins from diverse families exhibit active incorporation into assembling HIV-1 particles by means of an unknown mechanism. Interestingly, Murine Leukemia Virus envelope glycoprotein (MLV Env) is actively recruited to viral assembly sites in the absence of its C-terminal tail. To identify the region(s) of MLV Env required for efficient recruitment into assembling particles, we generated a large cell library (approximately $6x10^5$ cells) with each cell stably expressing a MLV Env viral amplicon with randomly mutagenized external membrane-proximal and membrane-spanning domains. The random mutagenesis targeted 50 amino acid positions beginning roughly 20 residues N-terminal to the predicted start of the membrane-spanning domain and terminating 10 residues into the cytoplasmic tail. We chose a mutation rate of 3% which correlates to approximately 4.5 nucleotide changes per unique MLV Env mutant amplicon. Using *in vivo* selection coupled with next generation illumina sequencing we generated comprehensive libraries of mutant glycoproteins which were 1) enriched in viral particles, 2) able to promote viral infection and 3) remained fusogenically active. We have identified several amino acid residues crucial for promoting viral infectivity, facilitating viral-cell membrane fusion, and for promoting Env incorporation into assembling viral particles. Interestingly, mutants defective for fusion localize to the N-terminus of the membrane-spanning domain in the sequence pattern SxxxTTxxST, closely resembling a known helical packing consensus sequence SxxxSSxxT which is thought to drive homo-oligomerization between transmembrane helices. We hypothesize that these residues may aid in stabilization of the transmembrane portion of the Env trimer and in transmitting conformational changes needed to facilitate viral-cell membrane fusion.

In addition to probing novel aspects of Env functionality, this selection strategy can be applied to various targets under diverse selection pressures and is particularly valuable in its ability to minimize difficulties associated with targets highly sensitive to traditional mutagenesis, such as the viral capsid protein. Coupling next-generation sequencing technologies with this *in vivo* selection approach allows for generation of large data sets capable of sampling entire selected populations, providing insight into previously unidentified features of viral protein biology.

F31. HERV-K Env Can Interfere with HIV-1 Infection

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Human endogenous retroviruses (HERVs) occupy a large portion of our genome. Most of these retroviruses are not functional, due to either epigenetic silencing or to the accumulation of lethal mutations; nevertheless, expression of HERV proteins has been extensively reported. Only some of the most recently acquired HERVs, such as the HERV-K (HML-2) group, maintain intact, coding open reading frames. Expression of HERV-Ks has been linked to different pathological conditions, including HIV infection.

We have recently profiled HERV-K expression in primary lymphocytes from healthy donors and are currently characterizing the impact of HERV-K protein expression on HIV-1 infection. We have tested 20 different HERV-K envelope sequences for their ability to produce full-length and post-translationally processed envelope proteins, and to interfere with HIV-1 infection.

Our results show that the Env of HERV-K (HML-2 HOM) greatly diminishes the infectivity of HIV. This effect is due to the combined inhibition of HIV production and direct reduction of viral infectivity. We have detected the Env of HERV-K (HML-2 HOM) in the plasma membrane of transfected cells and found that it can be specifically incorporated into HIV virions. This is the first report describing the functional interference of a HERV envelope protein on HIV infection.

BACK

F32. A Tyrosine-Based Motif in the HIV-1 Envelope Glycoprotein Tail Mediates Cell Type- and Rab11-FIP1C-Dependent Incorporation into Virions

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Lentiviruses such as HIV-1 encode envelope glycoproteins (Env) with long cytoplasmic tails (CTs) that include motifs mediating interactions with host cell trafficking factors. We demonstrated recently that Rab11-FIP1C/RCP is required for CT-dependent incorporation of Env into HIV-1 particles. Here, we employed viruses bearing targeted substitutions within CT to map the FIP1C-dependent incorporation of Env. We identified YW_{795} as a critical motif mediating cell type-dependent Env incorporation. Disruption of YW_{795} reproduced the cell type-dependent particle incorporation of Env that had previously been observed with large truncations of CT. A revertant virus bearing a single amino acid change near the C-terminus of CT restored wildtype levels of Env incorporation, Gag-Env colocalization on the plasma membrane, and viral replication. We next extended these findings to include analysis of a C-terminal fragment of FIP1C (GFP-$FIP1C_{560-649}$). This construct acted as a dominant-negative for WT Env trafficking by trapping Env within an enlarged endosomal recycling compartment (ERC). In contrast, YW_{795} mutant virus was able to escape trapping in the ERC by GFP-$FIP1C_{560-649.}$ These findings highlight the importance of YW_{795} in the cell type-dependent incorporation of Env, and support a model of HIV assembly in which FIP1C/RCP mediates Env trafficking from the ERC to the particle assembly site.

F33. Purified RSV Gag is Extended and Flexible and Binds to Membranes in a Cooperative Fashion that Depends on the SP Domain.

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Biochemical dissection of Gag-membrane interaction requires substantial quantities of purified and biologically active protein, which has not been reported for HIV-1 because of solubility problems due to myristoylation. In contrast, RSV Gag is not naturally myristoylated. We developed protocols for purifying milligram amounts of RSV Gag (without an epitope tag and minus the PR domain), along with truncation mutants missing either the NC or MA domains. This Gag protein is biologically active based on its ability to assemble into VLPs in vitro. The availability of tractable purified protein has facilitated biochemical experiments that previously have not been possible with any other Gag protein in a biologically relevant form. By size exclusion chromatography and small angle X-ray scattering, RSV Gag is flexible but adopts an overall elongated rod-like conformation, in contrast to un-myristoylated HIV-1 Gag, which is also flexible but adopts a folded-over, compact structure. Neutron reflectivity of RSV Gag bound at low ionic strength to a supported lipid bilayer shows that the protein adopts a compact structure on the membrane, which is most readily interpreted to mean that both the MA and NC domains interact with the membrane simultaneously. Using surface plasmon resonance (SPR), we measured binding constants for the interaction of RSV Gag and RSV MA to supported lipid bilayers. Gag binds 100-fold more tightly than does MA (0.2uM and 27uM, respectively). At physiological ionic strength, Gag proteins missing the NC domain or missing the MA domain bind membranes very weakly. Based on the Hill coefficients estimated by SPR, the tighter binding of Gag is most readily interpreted to be due to protein-protein interactions, which are dependent on the presence not only of CA but also of the spacer peptide (SP) between CA and NC, but are not dependent on the presence of NC. This finding suggests that Gag-membrane interaction is driven in part by Gag multimerization triggered by SP. The ability of membranes to support Gag multimerization is independent of the presence of nucleic acid, and is enhanced by PI(4,5)P2.

BACK

F34. Effect of Membrane Order and Charge on the Binding of Retroviral Gag Proteins to Membranes In Vitro

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The retroviral Gag polyprotein provides the principal driving force for virus assembly and budding from the plasma membrane (PM). Several principles govern Gag-membrane binding, including electrostatic and hydrophobic interactions, Gag multimerization, and recognition of both lipid head groups and acyl chains. It has been said that retroviruses "bud from rafts", but this concept remains ill-defined. Published in vitro experiments with giant unilamellar vesicles (GUVs) with two lipid phases do not discriminate between Gag-related proteins recognizing lipid order itself (Lo or Ld phases) or recognizing the phase with the highest negative charge. To better understand how Gag interacts with the PM, we have designed and purified fluorescent electrostatic sensor proteins, such as GFP with a C-terminal tail of 4, 8, or 12 basic residues. The liposome binding of these proteins was compared to that of purified RSV Gag. The anionic lipids PS and PI(4,5)P_2 contributed to the recruitment of these polycationic proteins, as expected. Cholesterol also enhanced the binding of the polycationic proteins to membranes with fixed PS concentrations, similar to what we have previously reported for RSV and HIV MA and Gag. Both the electrostatic sensor proteins and RSV Gag, which was fluorescently labeled at its C-terminus at an 11-residue epitope for phospho-pantetheinyl transferase, had similar responses to acyl chain order (Lo or Ld) and head group type. We have used fluorescence resonance energy transfer to create a partial lipid phase diagram to allow the percentage of PS in co-existing Lo and Ld phases to be calculated. On GUVs, the electrostatic sensor proteins bound to the PS-rich phase, independent of lipid order; similar analyses are now being done for Gag. We are developing fluorescence correlation spectroscopy (FCS) as a method to rapidly measure protein-membrane binding constants. We plan to use GUVs with lipid compositions similar to those of the PM inner leaflet to study Gag assembly.

BACK

F35. Line Tension and Bending Modulus for Models of the Cell Plasma Membrane

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Viruses are commonly believed to bud from rafts. Raft + non-raft behavior in cells can be modeled by liquid-ordered (Lo) + liquid-disordered (Ld) phase coexistence in plasma membrane models consisting of high-melting temperature lipid, low-melting temperature lipid, and cholesterol. For these coexisting phases, domain sizes range from a few nanometers to many microns, depending strongly on the low-melting lipid. The nature of this transition implies that interactions compete to determine domain size. The progression of domain morphology can be modeled as a competition between line tension and rigidity. We have systematically measured line tension vs composition in a range of lipid mixtures, and we have measured rigidity for several different coexisting Lo and Ld phases with increasing amounts of the α-helical transmembrane peptide GWALP23. In addition to its role in phase morphology, the rigidity of the respective phases is a quantity of interest in processes that deform the membrane, like virus budding. Our results show that the presence of this peptide component makes bending of the Lo phase more favorable than bending of the Ld phase.

F36. Membrane Composition and Protein Lipidation as Determinants of the Free Energy of HIV-1 Matrix Membrane Binding

Marilia Barros, Frank Heinrich, Hirsh Nanda, Siddartha A. K. Datta, Alan Rein, and Mathias Lösche

The N-terminal matrix domain of Gag, MA, is the key structural motif that mediates membrane binding. Several biophysical mechanisms are implicated in MA-membrane binding, including electrostatic interactions between a patch of basic amino acids and anionic lipids, hydrophobic interactions with MA's myristoylated N-terminus, and specific binding to phosphatidylinositolbisphosphate, $PI(4,5)P_2$, in the plasma membrane. Despite extensive efforts to delineate the individual contributions of those interactions to membrane binding, no consensus has yet been achieved. In particular, a quantitative rationalization of the fact that virus formation depends critically on Gag myristoylation is still lacking. Here we use solid-supported tethered bilayer lipid membranes (tBLMs) and surface plasmon resonance (SPR) spectroscopy to directly monitor protein binding as a function of concentration. At ionic strengths where both electrostatic and hydrophobic interactions contribute to membrane association, a comparative study of –myrMA and +myrMA shows that myristoylation increases MA membrane association significantly: $K_D \approx 5$ μM vs. ≈ 50 μM for –myrMA in the absence of PIP_2. That argues against the view that PIP_2 binding is essential for protein activation by myristoyl exposure at the plasma membrane and disagrees in quantitative terms with vesicle (LUV) flotation assays from which it was concluded that MA dimerizes for effective membrane binding. On the other hand, the free energy contribution of myristoylation to binding is much less than the insertion energy of a free myristoyl chain into a membrane. A number of effects can potentially contribute to this difference between free acyl chains and protein myristoylation: (1) an entropy reduction associated with protein membrane binding; (2) transfer of the myristoyl from its hydrophobic sequestration in the dissolved protein into the bilayer has a lower enthalpy change than transfer of a free fatty acid from aqueous solution; (3) myristoyl exposure from the protein may still be incomplete in the absence of PIP_2, or membrane insertion may remain incomplete for other reasons. While cholesterol and $PI(4,5)P_2$ further increase the affinity of +myrMA for the bilayer ($K_D < 1$ μM at 5% PIP_2 and 30% chol), the differences, $\Delta\Delta G$, between MA proteins with and without myristoylation decline with increasing affinities, emphasizing the role of entropic restriction of the protein's degrees of freedom when bound to the membrane.

BACK

F37. Investigation of Feline Immunodeficiency Virus Matrix Protein Assembly to the Plasma Membrane

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The human immunodeficiency virus (HIV) remains a global health concern. Drug resistance and non-compliance to current treatment demonstrate the necessity for novel therapeutics, but development of new treatment has been limited by the absence of an appropriate animal model. Because of strong resemblance between the feline immunodeficiency virus (FIV) and HIV, felines are a tenable animal model for HIV in humans. Previous work has shown that the matrix domain (MA) of the Gag polyprotein is essential for efficient viral assembly and release and that HIV MA binds to the plasma membrane via phosphatidylinositol-(4,5)-bisphosphate [PI(4,5)P_2]. While FIV MA has been shown to target PI(4,5)P_2, the mechanism by which FIV MA binds to PI(4,5)P_2 has yet to be characterized. This work investigates FIV MA assembly to the plasma membrane by utilizing liposomes as mimetic membranes. Interaction between FIV MA and liposomes is characterized by means of nuclear magnetic resonance spectroscopy (NMR). Because the size of liposomes exceeds the detection limit of NMR, observation of the free (unbound) protein signal is monitored. Characterization of FIV MA membrane targeting will allow for comparison to that of HIV MA, potentially giving rise to a plausible drug target to advance current HIV treatment.

BACK

F38. Modes of Interaction of Retroviral Proteins with Lipid Bilayers: A Molecular Dynamics Simulations Study

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Rous sarcoma virus (RSV) serves as a model retrovirus used to study the association of the structural protein, Gag, of enveloped viruses such as HIV with the plasma membrane (PM). Gag-PM interactions are an important step in the viral lifecycle, and understanding the mechanism of these interactions could facilitate the development of retroviral therapies. *In vitro* and *in vivo* experiments have provided important results regarding the binding of RSV Gag to model membranes, but a detailed molecular picture of the protein-bilayer interaction is missing. Here we present molecular dynamics simulations performed on the membrane-binding domain (MBD) of the N-terminal MA protein of RSV Gag in the presence of a lipid bilayer composed of neutral and charged lipid species. We analyze the dynamics of the protein, its modes of interaction with the model membrane, and the corresponding effects on bilayer structure. The application of the simulations to the analysis of small angle neutron scattering data is discussed.

BACK

F39. Three-Dimensional Structural Characterization of HIV-1 Tethered to Human Cells by Cryo-electron Tomography

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Tetherin is a cellular restriction factor that inhibits HIV-1 release by mechanically linking viruses to the plasma membrane. How tetherin molecules engage HIV-1 virions during assembly, budding and release is not clearly defined. To gain insight into this process we directly visualized HIV-1 virus like particles (VLPs) and virions tethered to human cells by cryo-electron tomography. Rod-like filaments which we refer to as "tethers" bound to HIV-1 connected to each other and to the plasma membrane were observed. These tethers were not seen on released virions produced by cells transfected with tetherin C3A, a previously characterized tetherin mutant, which does not form stable homodimers and does not inhibit HIV-1 release. The arrangement of the tethers relative to the immature Gag lattice and mature conical-core of HIV-1 virions was random. However when present as multiple copies the tethers congregate into clusters which were sometimes situated on opposite poles of the mature virion. Three-dimensional distance measurements support the extended tetherin model, in which the tetherin coil-coiled ectodomains adopt an extended conformation and are oriented perpendicular to the viral and plasma membrane. Interconnected HIV-1 virions were arranged in linear fashions, which resemble beads on a string, branching between tethered virions was rarely observed.

BACK

F40. Cellular Release and Uptake of Designed Enveloped Nanoparticles

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Viruses like HIV-1 can assemble and bud from producer cells and deliver cargoes into the cytoplasms of new target cells. In principle, these activities have potential therapeutic applications but current synthetic technologies are often limited by inefficiency and lack of flexibility, whereas viral vectors can be limited by safety concerns, immunogenicity, and difficulties in packaging non-nucleic acid cargoes. As a test of our understanding of the requirements for viral particle assembly, envelopment, budding, and target cell re-entry, we have created designed nanoparticles that spontaneously assemble into different platonic solids, including dodecahedra and icosahedra. These self-assembling proteins were expressed in human cells and used to test the requirements for particle egress and entry.

Designed nanoparticles could be targeted to membranes through the addition of the HIV-1 MA myristoylation signal and other membrane targeting domains. Membrane-associated nanoparticles could be released from cells through the addition of the $p6^{Gag}$ region of HIV-1, and particle release was dependent upon the presence of both the TSG101-binding PTAP and ALIX-binding YPXL motifs, demonstrating that particle release required recruitment of the cellular ESCRT machinery. EM analyses revealed that assembled articles were released predominantly within enveloped extracellular vesicles, rather than as individual particles. A Vpr-β-lactamase fusion protein could be specifically incorporated via a non interaction between the Vpr and $p6^{Gag}$ proteins, and expression of VSV-G protein allowed the particles to enter new target cells, as assayed by β -lactamase activity. Thus, we have designed icosahedral nanoparticles that can self assemble in mammalian cells, bind membranes, exit cells in an ESCRT-dependent fashion, and enter new target cells to deliver enzymatic cargoes. This system will be used as a starting point for optimization of synthetic delivery systems based on the principles employed by enveloped and non-enveloped viruses.

BACK

F41. Studies of HIV-1 Maturation *in vitro*

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HIV-1 virions assemble as immature particles containing $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ polyproteins. During and following HIV-1 budding, the viral protease (PR) cleaves Gag into its component domains, the matrix protein (MA), capsid protein (CA), and nucleocapsid protein (NC), leading to a dramatic structural rearrangement within the virion and the formation of a functionally distinct, mature infectious virion. Previous studies have led to two distinct models for the formation of HIV-1 mature virion core. In the disassembly-reassembly model, the immature lattice disassembles following PR cleavage, generating a pool of soluble CA molecules from which a mature core reassembles *de novo*. By contrast, the displacive transformation model posits that mature capsid formation involves a direct, non-diffusional remodeling of the immature Gag lattice to the mature capsid lattice. To study the structural rearrangements in HIV-1 maturation and the sequence of structural changes that occur, we simulated HIV-1 maturation *in vitro* by digesting detergent-treated immature particles and assembled Gag virus-like particles (VLPs) with HIV-1 PR and monitored the corresponding structural changes using cryoEM. Processing of $Pr55^{gag}$ by PR was accurate and efficient in the reaction. Biochemical analysis of the PR-treated particles revealed solubilization of MA, CA, and NC in a time- and dose-dependent manner. However, cryoEM images of PR-treated Gag VLPs showed formation of assemblies resembling mature capsid structures, suggesting the possibility of reorganization of the lattice rather than complete dissociation and reassociation of the subunits during particle morphogenesis. We also investigated the effects of BVM and PF74, as well as Gag cleavage mutants, on particle maturation.

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F42. MAS NMR Characterization of HIV-1 Maturation Intermediates

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HIV-1 viral maturation is an attractive target for therapeutic intervention. Gag is the key structural protein of the immature HIV virion, comprising ~ 50% of its mass.[1] During viral maturation, Gag is cleaved into its constituent domains, which undergo conformational changes to form a mature infectious virion. Atomic-level understanding of HIV-1 maturation mechanism remains incomplete, including the structure of immature Gag and the interactions of Gag and maturation intermediates with maturation inhibitors. We present investigations of Gag assembled into immature viral like particles (VLPs) as well as assemblies of the maturation intermediates CA-SP1 and CA-SP1-NC, by magic angle spinning (MAS) NMR spectroscopy. We demonstrate that these assemblies are amenable to detailed structural characterization by MAS NMR. Our current high-quality results for Gag VLPs set the stage for the characterization of Gag secondary structure, dynamics, and interactions with RNA. Our results in CA-SP1 T8I mutant (which mimics the effect of the maturation inhibitor Bevirimat[3]) indicate the SP1 peptide forms a stable structure, in contrast to wild type CA-SP1 assemblies, where SP1 is a dynamic random coil.[4] We further demonstrate experimental conditions that yield high-resolution MAS NMR spectra of RNA and present partial resonance assignments in U-^{13}C,^{15}N-Iron Responsive Element RNA, a critical step toward the characterization of large RNA structures in the context of viral systems.

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F43. Development of Potent and Broadly Active "Second-Generation" HIV-1 Maturation Inhibitors; Insights into Gag Structure and Function

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A betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor, acts by blocking a late step in protease-mediated Gag processing: the cleavage of the capsid-spacer peptide 1 (CA-SP1) intermediate to mature CA. BVM was shown in multiple clinical trials to be safe and effective in reducing viral loads in HIV-1-infected patients. However, polymorphisms in the SP1 region of Gag (e.g., SP1-V7A) lead to variable response in some BVM-treated patients. The reduced susceptibility of SP1-polymorphic HIV-1 to BVM led to the discontinuation of its clinical development. To overcome this problem, we carried out an extensive medicinal chemistry campaign to develop "second-generation" maturation inhibitors based on the BVM scaffold. We identified a set of BVM derivatives that are not only markedly more potent than BVM against prototypical clade B HIV-1 but also show robust antiviral activity against clade B isolates with SP1 polymorphisms and against a panel of non-clade B primary isolates in T cell lines and PBMC. IC_{50}s against WT NL4-3 and the SP1-V7A polymorphic HIV are in the low-nM range.

To define the target and mechanism of action of the second-generation maturation inhibitors, we selected for compound-resistant viruses. In addition to identifying mutations around the CA-SP1 cleavage site, we also selected for the single-amino-acid mutation CA-P157A, located in the major homology region (MHR) of CA. Remarkably, the P157A mutant was resistant to not only BVM and the second-generation BVM analogs but also to the structurally distinct maturation inhibitor PF-46396. In contrast to our previously described PF-46396-resistant MHR mutants that were profoundly compound-dependent, we found that P157A replicates efficiently in either the presence or absence of compound. We have used multiple biochemical, structural, and virological approaches to evaluate the properties of resistant mutants in terms of Gag assembly, virus maturation, and replicative fitness. These studies provide novel insights into the structure and function of CA (including the enigmatic MHR) and the CA-SP1 boundary region. In addition, the identification of BVM analogs that are highly potent against a wide range of HIV-1 isolates containing polymorphic SP1 sequences suggests that clinical development of HIV-1 maturation inhibitors should proceed.

F44. Development of Novel HIV Maturation Inhibitors against HIV Subtype C

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One of the major problems faced during anti-retroviral treatment of HIV patients is evolution of drug-resistant viruses. Hence, identification of new antiviral targets continues to be a high priority for development of HIV therapeutics. Maturation inhibitors represent a new underdeveloped class of antiretroviral agents that block virus maturation not by targeting the PR enzyme itself but by binding the target of PR (Gag) and blocking a specific step in Gag processing.

The first-in-class maturation inhibitor Bevirimat (BVM) was found to be ineffective against a subset of HIV-1 isolates in vitro as well as in clinical trials. The failure of the drug against these strains was attributed to polymorphisms mainly present in the CA-SP1 region of HIV-1. To overcome this problem, a number of BVM derivatives (referred to as "second generation" maturation inhibitors) were generated. The BVM analogs were found to be more effective than the parental compound against both HIV subtype B and HIV subtype C. Another structurally distinct maturation inhibitor PF-46396 was also found to be potent against HIV subtype C. Efforts are underway to select for compound resistant viruses, which will guide in identifying the putative binding pocket of these compounds on HIV Gag and help in clinical development of these compounds.

BACK

F45. Exploring the Interdependence of Sub-Sites in the Active Site of HIV-1 Protease

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HIV-1 protease recognizes and cleaves a series of non-homologous sequences as specific substrates. These diverse substrates adopt a conserved shape when bound to the protease due to dynamic interdependence of different sub-sites within the protease active site. This sub-site interdependence, observed in both substrate- and inhibitor-bound protease structures, has not been exploited for the design of new inhibitors. We previously tested the sub-site interdependence with designed pairs of compounds by changing the ligand size at three positions (Shen *et al. ACS Chem Biol*, 2013, 15, 2433). To further explore the interdependence of different sub-sites within the protease active site, we have designed and synthesized a series of new inhibitors using combinations of ligands with varying sizes and flexibility. Inhibitors incorporating relatively inflexible moieties at all four P1/P1′ and P2/P2′ positions exhibited poor activity against wild-type protease and lost significant activity against drug-resistant variants. However, when one or more of the groups were highly flexible, the inhibitors showed highly potent activity against wild-type protease and retained potent activity against resistant variants. Interestingly, in some cases the flexible ligand was at P2 position while most of the mutations in the protease variants tested cluster around the S1/S1′ regions. These data suggest that the interdependency of different sub-sites within the protease active site can be exploited to optimize the activity of protease inhibitors against drug-resistant variants.

F46. Construction of a SHIV Theoretically Capable of Modeling HIV-1 Protease Inhibitor Potency in Non-Human Primates

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In order to develop a system to test HIV-1 protease (PR) inhibitors in non-human primates, we generated a SHIV construct based on the SHIV-pr design of Ishimatsu, M. et al. (2007). SHIV-pr was constructed by replacement of the *pro* gene (encoding for PR) in an SIVmac infectious molecular clone (mac239 strain) with an HIV-1 Subtype B *pro* gene (NL4-3 strain). The resultant virus was reported to be replication competent *in vitro* and in rhesus macaques, but had delayed growth characteristics. We hypothesized that the HIV-1 PR is unable to cleave SIVmac cleavage sites as efficiently as the HIV-1 cleavage sites; this is suggested by the fact that there is the up to 80-fold lower potency of HIV-1 PR active site competitive inhibitors with HIV-2 PR (Brower, et al., 2008), a virus of the same lineage as SIVmac. In order to optimize this system, we designed a new clone (SHIV*pro*+) that additionally has the GagProPol cleavage sites (P4-P4' residues) substituted for HIV-1 sequence residues similar to the subtype B consensus sequence (Torrecilla, E. et al., 2014). We theorize that matching the PR with its natural substrate recognition sequence should improve growth characteristics of this SHIV. We present a novel method for constructing SHIV*pro*+ by utilizing sequential enzymatic assembly of 10 PCR products with overlapping ends containing the engineered mutations. Up to 4 products were assembled at a time, the assemblies were enriched by PCR, and this method was iteratively repeated until the half-genome was reconstructed in a cloning vector. Fortuitous mutations were corrected by PCR and the half-genome was fully sequenced. Preliminary data on the *in vitro* growth characteristics of this construct, SHIV*pro*+, will be presented.

F47. Hydration and Dynamics in Inhibitor-Bound HIV-1 Protease using NMR Spectroscopy

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Human immunodeficiency virus type-1 protease undergoes mutations throughout the enzyme upon the selective pressure of protease inhibitors. These mutations can, over time, weaken inhibitor binding and change the dynamics of the enzyme. In particular with potent and robust inhibitors, such as Darunavir (DRV), drug resistance develops through combinations of mutations and complex pathways. Understanding the molecular mechanisms underlying drug resistance is important for formulating in drug design. Our group characterizes dynamic components of the protease-drug interaction mainly using nuclear magnetic resonance (NMR) spectroscopy. In our current studies, we are determining the roles that water and dynamics play in the binding of DRV and other novel protease inhibitors.

Supported by: NIH P01 GM109767

F48. Exploring Surface Sites on HIV Protease as Targets for Inhibitors: Promises and Failures

Tiefenbrunn, T.[1], Forli, S.[1], Happer, M.[2], Gonzalez, A[3]., Baksh, M. M.[4], Chang, M. W.[5], Tsai, Y.-S.[3], Lin, Y.-C.[2], Perryman, A. L.[1], Rhee, J.-K.[4], De Vera, I.[6], Kojetin, D.[6], Torbett, B.E.[5], Olson, A. J.[1], Soltis, M.[3], Elder, J. H.[2], Finn, M. G.[4], Stout, C. D.[1]

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A crystallographic fragment-based screen against PR identified two surface sites, the flap site and the exosite, that are potentially important for protein-protein interactions with Gag and are alternatives to the active site for drug design (1). Cocrystals of indole-6-carboxylic acid and 3-indolepropionic acid binding in the flap site were obtained with apo PR with closed flaps (2). A larger BSI phase change was observed for binding of these fragments to apo PR vs. pepstatin:PR, indicating a larger conformational change upon compound binding in solution, suggesting that compound binding forces the flaps closed.

To find additional hits, a brominated fragment library was screened against a novel $I4_122$ form of W6A PR. Of a library of 68 compounds, one hit was identified in the flap site and one in the exosite. An additional 9 bromine binding sites were found in each site with a strong Br anomalous peak but weak compound density. Br sites provide insight into the nature of the binding pockets, and, for the flap site, showed a clear direction for fragment expansion.

Based on prior fragment hits, computational studies in the Olson group identified 1-(4-methylphenyl)sulfonyl-3-(1,3-thiazol-2-yl)urea (C6) as a potential exosite binder. C6 binds to PR as indicated by DSF and BSI assays with 6.5 ºC stabilization and 0.96 +/- 0.11 μM affinity, respectively. Additionally, it is weakly inhibitory in an enzymatic cleavage assay (56% inhibition of protease at 1 mM). C6 has been co crystallized in two crystal forms of TL-3:PR, conserving interactions observed for other fragments. The observed binding mode of C6 involves a portion of the molecule being solvent exposed, suggesting modes for fragment evolution to more closely bind in the exosite pocket of PR.

Based on these results, a new series of compounds was tested for binding to protease via BSI. Of this series, HIVE9 showed the most promising results for binding to the apo-protease, with an affinity of ~1 μM. However, both NMR and cocrystallization results suggest that HIVE9 is binding in the active site rather than the exosite of protease. In one crystal structure with TL-3:PR, HIVE9 was observed to bind in an alternative site on the surface of HIV protease, but given the NMR results, it is likely that the active site binding is what is responsible for the observed BSI data.

F49. Implications of Hinge Region Natural Polymorphisms on HIV-1 PR Structure, Dynamics and Drug-Pressure Selected Evolution

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HIV-1 protease (PR) serves as an important drug target because its inhibition generates immature non-infectious virions. However, drug-pressure selected mutations, along with natural polymorphisms, can lead to drug resistance to HIV-1 protease inhibitors (PIs). As “guards” for controlling the logistics activities of substrates and drugs into/out-from catalytic pocket, HIV-1 PR flap adopts different conformations such as closed, semi-open and wide-open states (1). The flap conformations are closely associated with the enzyme kinetics and drug inhibition parameters, (2) which justify the significance of the investigation on HIV-1 PR flap conformations. As was suggested in our previous study (3), a new flap conformation, curled-open, could be identified in constructs carrying natural polymorphisms including E35D, and consequently, these polymorphisms could induced a higher protein backbone dynamics comparing with wild-type subtype B construct characterized by nuclear magnetic resonance (NMR) spectroscopy. In the present study, we will discuss the molecular mechanism of E35D natural polymorphism on influencing protein structure and dynamics in presence/absence of other natural polymorphisms by a combination of double electron-electron resonance (DEER), NMR spectroscopy and X-ray crystallography.

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F50. Mapping The Fitness Landscapes of Drug-Resistance in HIV Protease

Jeffrey I Boucher[1], Sook-Kyung Lee[3,4], Ronald Swanstrom[2,3,4], Celia A Schiffer[1], and Daniel NA Bolon[1]

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The evolution of drug resistance is a pressing issue in human diseases ranging from bacterial and viral infections to cancer. Chemical intervention applies the selective pressure necessary for the fixation of resistant variants. Current efforts to determine the resistance potential of drug targets rely on stochastic experiments, such as adaptive laboratory evolution and the analyses of clinical isolates. Systematic mutational scanning provides a promising alternative by comprehensively mapping the resistance landscape of a protein to actively inform preclinical drug design. HIV protease is an ideal model system to evaluate the scanning approach due to the theoretical potential for circulating viral populations to have fully explored all possible resistance mutations and the wealth of sequenced resistant isolates. To determine the local fitness landscape of HIV protease, we are generating a library of all single amino acid substitutions and will evaluate each mutation's effect on resistance to nine clinically approved protease inhibitors. Because highly resistant clinical isolates feature multiple mutations, we will analyze a combinatorial library of all single resistance mutations along with known compensatory mutations to identify the key epistatic interactions that shape the evolutionary pathways leading to high resistance. The results of these comprehensive screens will be compared to the database of resistant clinical isolates to critically evaluate the potential of systematic mutational scanning to map resistance potential and inform preclinical drug design.

F51. Analyses of Accessory Mutations in HIV-1 Protease Reveal Interdependent Mechanisms of Resistance

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Background: The contribution of accessory mutations in conferring drug resistance to HIV-1 protease inhibitors has been studied in great detail but remains elusive. Discerning the synergy of both types of mutations to sustain resistance to highly potent inhibitors while perpetuating viral longevity is the basis of this study. Kinetic and structural analyses of four accessory mutations have revealed possible interdependent mechanisms of mutations beyond the active site and their role in drug resistance.

Methods: In this study, four HIV-1 protease mutants, L76V, L90M, V32I, and V32I+L33F, were characterized for their impact on Darunavir inhibition. Each of the mutants was first tested for DRV susceptibility using a FRET based kinetics. Each of the four mutants were then co-crystallized with DRV and characterized. Using the trajectories from 10ns molecular dynamics simulations the root mean square deviations and fluctuations for each mutant complex was assessed. The trajectories from the simulations were also used to calculate distances for inter and intra- monomeric active site residue pairs to determine the bearing of accessory mutations on active site residues.

Results: Although the four mutations observed in this study do not directly lie within the active site or bind to DRV they each impact various aspects of DRV inhibition including a 22.5 fold change in K_I exhibited by the V32I+L33F double mutant. Analyses of the co-crystal structures reveal that while no major changes can be observed in both overall structure and static Cα distances, there are significant changes in van der Waals contacts upon the introduction of L33F. Results from molecular dynamics simulations suggest that each mutation affects DRV binding via a network of intra-monomeric main chain polar contacts to reduce protease susceptibility to DRV by altering the conformations of residues within the active site.

Conclusions: The interdependent structural and dynamic mechanisms of resistance employed by the accessory mutations in this study account for loss of susceptibility to one of the most potent HIV-1 protease inhibitors.

F52. Understanding the Dynamics of Drug Resistance Mutations using HIV-1 Protease as a Model System

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Drug resistance is a global health problem and the leading cause of treatment failures. Current drug design strategies ignore the evolution of drug resistance and therefore, strategies to combat resistance are reactionary. We need prospective drug design guidelines to move forward and prevent drug resistance. Structure-based drug design (SBDD) offers exciting ways to examine resistance at the atomic level by providing unique insights of protein-ligand-water interactions. However, SBDD provides only a static representation, the dynamics of the target system remain hidden. Elucidating protein dynamics is of vital importance to combatting drug resistance, as resistance mutations are known to alter system dynamics. Using HIV-1 protease as a model system, I am evaluating the impact drug resistant mutations have on the molecular dynamics of protein-ligand-water interactions. We have developed a library of darunavir analogs that have been shown to have sub-nanomolar potency *in vitro*. Our darunavir analogs have been used as selective agents for viral passaging and passages were subsequently deep sequenced. Using this schema we can determine pre-clinically specific resistance mutations to HIV-1 protease that arise due to darunavir analogs. Starting with high resolution crystal structures solved in our laboratory, long time scale (100 ns) simulations were performed using these resistance mutations and compared to wild-type trajectories. Analysis of molecular dynamics trajectories reveals differences in van der Waals contacts, hydrogen bonding and water structure between wild-type and mutant trajectories. By understanding these differences I aim to unearth underlying patterns and ultimately work towards building a universal model that can be used to design "resistance-proof" inhibitors.

F53. Selection to Confirm Novel Resistance Pathways to Potent New HIV-1 Protease Inhibitor

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We previously carried out selection of HIV-1 growing in CEMX174 cells for resistance to UMASS 1-10, HIV-1 protease inhibitors which are derivatives of DRV. We also included DRV and a no-inhibitor control. We originally used a mixture of 26 variants, each containing single PI-resistance mutation, as the starting virus. Deep sequencing analyses performed at four time points revealed three unique resistance patterns associated with specific structures in the inhibitors. To confirm these results, we are carrying out a 2nd selection with several modifications. First, we used wild type virus, NL-CH derived from NL4-3, as the starting virus instead of using a mixture of 26 variants. Second, we used 1 nM as an initial inhibitor concentration based on EC50 values determined in a TZM-bl cell-based infectivity assay. EC50 values of the UMASS 1-10 including DRV determined in a TZM-bl cell-based infectivity assay ranged from 2 nM to 9 nM. Currently, the starting NL-CH virus has undergone 20 passages where drug concentrations range from 3 nM to 240 nM. We observed that the inhibitors fall into three groups depending on the rate of increasing drug concentration that still allows robust virus replication in the culture: rapid (UMASS-6, 7, and 9), slow (UMASS-4, 7, and 10), and poor (UMASS-1, 2, 3, and DRV). Inhibition of virus replication was apparent as the drug level reached the EC50. We performed bulk sequencing using cell pellet DNA from the passage with the drug concentration around 10 nM. We detected 1-3 mutations in the protease region, V82I (UMASS-2, 4, and 7), A71V (UMASS-5), I50V/A71V (UMASS-6 and 9), A71V/V82I (UMASS-10), and M46I/I50V/A71V (UMASS-8). We did not detect any mutations from the cultures of UMASS-1, 3, and DRV. Resistance data from passage 25 will be included.

F54. Understanding Antiviral Drug Resistance and Broadly Neutralizing Antibodies in Influenza

Kristina Prachanronarong, Zhen Zhang, Ellen Nalivaika, Aysegul Ozen, Safak Yilmaz, Kelly Thayer, Konstantin Zeldovich, Jennifer Wang, Wayne Marasco, Robert Finberg, and Celia Schiffer

University of Massachusetts Medical School, Worcester, MA, USA

Although vaccines and antiviral medications are available for influenza prophylaxis and treatment, influenza infects over 24 million people in the United States each year, causing over 200,000 hospitalizations and 40,000 deaths. Influenza neuraminidase (NA) is a viral sialidase on the surface of the influenza virion that releases budding virus from the surface of infected cells, and it is a primary antiviral target in the treatment of influenza. Two subtypes of NA predominate in humans, N1 and N2, but different patterns of drug resistance have emerged in each subtype even though the active sites of these NAs are highly homologous. Our laboratory studies drug resistance in viral proteases, and we found that natural substrates of HIV and hepatitis C proteases fill a conserved enzyme-specific volume when bound to the active site, known as the *substrate envelope*. Furthermore, primary drug resistance mutations occur where inhibitors protrude beyond the *substrate envelope,* and our laboratory established this paradigm as the *substrate envelope hypothesis.* To provide a framework for understanding the structural basis of subtype specific drug resistance mutations in NA, I used molecular dynamics simulations to define *dynamic substrate envelopes* for NA and to determine how different patterns of drug resistance have emerged in N1 and N2 NA. In addition, influenza hemagglutinin (HA) is a protein on the surface of the influenza virion that is responsible for cell entry and membrane fusion, and it is a primary target of neutralizing antibodies against influenza. Novel broadly neutralizing antibodies (BnAbs) against the stem region of HA have been described and inhibit several influenza viral subtypes. We solved two crystal structures of Fab fragments that are important for understanding the structural basis of antibody binding for specific influenza BnAbs.

F55. Structural and Thermodynamic Investigation of the Role of Macrocyclization in Hepatitis C Virus Protease Inhibitor MK-5172's Potency and Resistance Profile

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Background: Structure guided drug design has been a powerful tool for the discovery and development of inhibitors to the viral HCV NS3/4A protease. The NS3/4A protease inhibitors (PIs) have benefited from extensive optimizations in the common P4 capping, P3-P1' peptidomimetic scaffold and various macrocyclization states. However, neither structural nor thermodynamic information have been presented evaluating the benefits of different protease macrocyclization states in the context of resistance. MK-5172 is extremely potent against the wildtype (WT) enzyme, while subverting resistance to R155K and D168A due to packing against the protease catalytic triad, this inhibitor is susceptible to A156T due to a strong steric clash.

Methods: A combination of standard protein crystallographic, molecular dynamic, kinetic and thermodynamics methods were applied to the protease domain of HCV NS3/4A WT and A156T resistance variant. Detailed structural analysis, including fit within the substrate envelope and dynamic inhibitor envelope, was applied to a series of MK-5172 macrocyclic analogs and compared to the parent MK5172. This data was compared with the thermodynamics of inhibitor binding.

Results: A crystallographic, biochemical and thermodynamic characterization of the role of macrocyclization in drug resistance will be presented for the HCV NS3/4A PI MK-5172 and a series of analogs. Through a series of crystal structures in complex with WT and A156T HCV protease variants compared with thermodynamic data we provide atomic level insight into the inhibitor's unique binding mode and how the macrocyclization impacts susceptibility to drug resistance.

Conclusions: Through leveraging evolutionarily restricted regions in the HCV protease robust inhibitors can be designed to increase the barrier to resistance.

1. Evaluating the Role of Macrocycles in the Susceptibility of Hepatitis C Virus NS3/4A Protease Inhibitors to Drug Resistance. Ali A, Aydin C, Gildemeister R, Romano KP, Cao H, Ozen A, Soumana D, Newton A, Petropoulos CJ, Huang W, Schiffer CA. ACS Chem Biol. 2013 Jul 19;8(7):1469-78. doi: 10.1021/cb400100g. Epub 2013 May 1.
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BACK

F56. Exploring Tryptophan Stacking Capabilities in the HIV-1 Nucleocapsid Protein: Insights From Docking Calculations

Victor H.F. Bernardes[1,2], Raphael E.F. de Paiva,[1,3], Maria D. Vargas[2], Pedro P. Corbi[3], and Nicholas P. Farrell[1]

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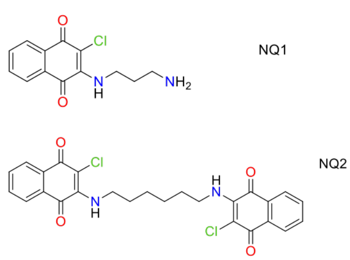
A critical feature of the HIVNCp7-nucleic acid interaction is the involvement of the aromatic amino acids tryptophan (Trp) and phenylalanine (Phe) in the molecular recognition of RNA/DNA. The mutation of even one of these residues significantly decreases NC's nucleic acid chaperone activity, and correlates with inhibition of viral replication (1). Metallation of nucleobases, as with protonation and alkylation, enhances *π-π* stacking interactions with tryptophan, and we are actively engaged in design of metallated-nucleobases targeted to tryptophan and interrupting the NCp7-RNA interaction (2). To expand the ligand base we have used the CLC Drug Discovery Workbench docking software to examine docking of potential naphthoquinone ligands to tryptophan in NCp7 C-terminal finger (PDB entry 1MFS) (3). In this contribution we demonstrate this approach emphasizing mono- and bis- alquil linked naphthoquinones (**I**: NQ1 and NQ2, respectively). NQ2 bridge is flexible enough for a "sandwich" stacking with tryptophan take place (**II**), resembling the interaction of some dinuclear Pt(II) compounds with guanine, as recently reported by us (**III**) (4). Initial fluorescence results using the C-terminal HIVNCp7 zinc finger confirm the concept. The initial results will be presented.



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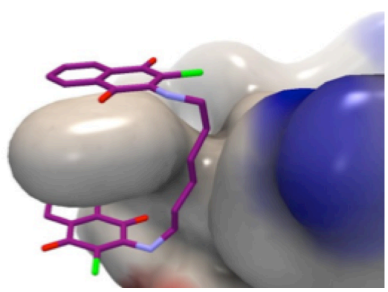

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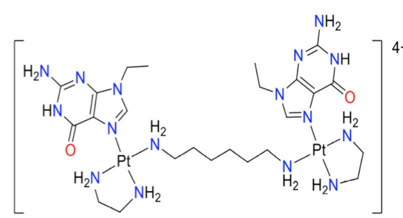

III

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BACK

F57. HIV-1 Nucleocapsid-RNA Antagonists Based on Zn-Ejecting Metal Complexes

Raphael E. F. de Paiva[1,2], Camilla Abbehausen[1], Sarah Spell[2], Samantha Tsotsoros[2], Erica J. Peterson[2], John B. Mangrum[3], Daniele Fabris[3] and Nicholas P. Farrell[2]

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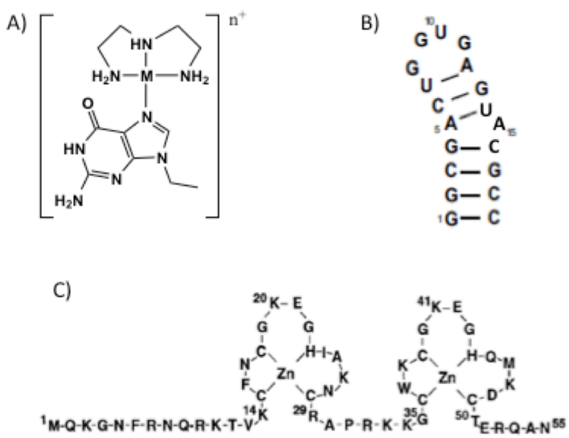
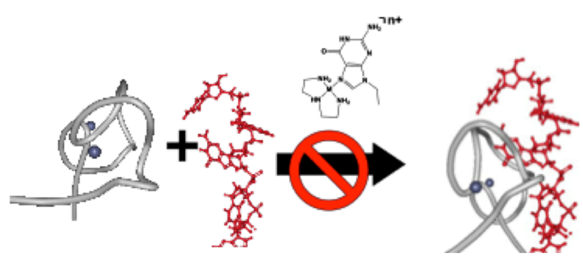
We have been exploring the potential of electrophilic metal complexes as inhibitors of the HIVNCp7 nucleocapsid protein. Protonation, alkylation or metallation of nucleobases enhance stacking with aromatic amino acids (1-3). The molecular recognition motif for HIVNCp7-RNA is now well detailed, with Trp and Phe interacting with nucleobases through both H-bonding and π-stacking where the indole ring of the W37 tryptophan residue is inserted between adjacent C and G bases and stacked on the latter. A metallated purine can stack with tryptophan followed by electrophilic attack on cysteines and Zn^{2+} ejection. In this contribution we show evidence by fluorescence spectroscopy, CD, ESI-MS and gel shift experiments that small molecules based on metallated purines are effective inhibitors of the HIVNCp7-SL2RNA interaction representing a new chemotype for zinc finger-nucleic acid antagonists. We also present structure-activity relationships for stacking interactions based on modified nucleobases.



Structures of A) $[M(dien)(9\text{-}EtG)]^{n+}$(M=Au, n= 3, Pt, n=2); B) SL2RNA (SL2) and C) NCp7 (NC).



Metal-nucleobase compounds as potential NC-Nucleic Acid antagonists.

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BACK

F58. Predicting Metalation Sites on HIV-1 Nucleocapsid Protein: A Bioinformatics Approach Using Blue Star Sting

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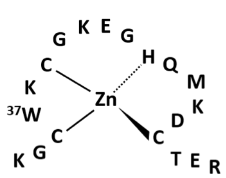
The HIV-1 nucleocapsid protein composed of two Cys_3His zinc finger domains is essential for nucleic acid sequence recognition and for viral encapsidation during the viral replication cycle. To this end, we have previously reported on the antagonist activity of the new chemotype [M(dien)(9-EtGuanine)]$^{n+}$ (M = Pt(II), n=2, M= Au(III), n=3].[1,2] The design is predicated on the molecular recognition of the essential Trp37 residue of the peptide by metallated purine.[2] Further displacement of purine can occur with covalent fixation to a cysteine residue and zinc ejection. A question that arise when designing new electrophilic metal complexes for zinc ejection is how selective the metalation is in terms of site-specificity and how to design ligands with enhanced stacking capabilities.

In this contribution, we have used the bioinformatics tool Blue Star Sting (http://www.cbi.cnptia.embrapa.br/SMS/)[3] to compare the Zn-bound residues of NCp7(F2) (PDB entry 1MFS, NMR solved structures).[4] Table 1 shows the numerical values for the three descriptors: (i) electrostatic potential at the side chain's last heavy atom, (ii) solvent accessibility area and (iii) sponge, the density of atoms within a defined sphere. Among the cysteines, Cys39 and Cys49 have the lowest electrostatic potential at the thiolate group.[5] Considering the susceptibility to electrophilic attack and solvent accessibility area of each residue, it is possible to identify Cys39 as the most accessible residue followed by His44. Proof-of-principle of the utility of this approach was found using [Au(I)] compounds related to auranofin, itself of potential interest as a HIV drug.



Residue	E. Potential @LHA / kT e^{-1}	Accessibility / Å^2	Sponge
Cys36	-18.3	2.42	0.73
Cys39	-33.35	52.88	0.53
His44	-90.22	41.04	0.69
Cys49	-35.91	15.17	0.66

Table 1: Java Protein Dossier descriptors for the Zn-bound residues found at NCp7 C-terminal finger.

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BACK

F59. High Resolution Structure of HIV-1 Capsid Assembly

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The human immunodeficiency virus 1 (HIV-1) mature capsid protein (CA) contains two structural domains that are connected by a flexible linker and assembles into a distinct cone shaped capsid that encloses the viral genome. The mature HIV-1 capsid plays a major role in regulating the early stages of HIV-1 replication by interacting with many host factors including CypA, CPSF6, MxB, TRIM5α and TRIM-Cyp. We have previously determined the CA tubular assembly to 8.6 Å using cryoEM and built an all-atom computer model of the complete capsid by large scale molecular dynamics simulations. Yet, the precise chemical environment essential for assembly and host factor interaction remain elusive. Exploiting the recent advance in direct electron detection and overcoming many technical challenges associated with large and flexible assemblies, we have now independently reconstructed three cryoEM density maps of CA tubular assemblies from (-12, 11), (-13, 12) and (-13, 11) helical symmetries at 4.8, 5.6 and 5.6 Å resolution, respectively. The 4.8 Å structure clearly resolves bulky side chain densities, helix grooves and connecting loops, which enables accurate molecular modeling by computer simulations. For the first time, the flexible linker and the major homology region are clearly visualized in an assembly context, providing insights on their critical roles in capsid assembly and maturation. Comparing the detailed assembly interfaces in the three independent structures further shed light on the plasticity of the CA molecule and the mechanism by which CA molecules accommodate the variable curvature of mature conical capsid.

F60. Structural Studies of HIV-1 Capsid Protein Assemblies by Sensitivity Enhanced Magic Angle Spinning NMR

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In human immunodeficiency virus (HIV-1), ~1200 copies of the 26.6 kDa capsid protein (CA) assemble to form cone-like structures that can enclose the viral RNA genome during viral maturation (1,2). An important step in this maturation process, which facilitates the condensation of CA protein into capsid, is the cleavage of the SP1 peptide from the CA-SP1 maturation intermediate. The mechanism of the SP1 cleavage including the conformation of the SP1 peptide in CA-SP1 is under investigation. Magic angle spinning (MAS) NMR methods can provide detailed atomic-level information to elucidate the structure and dynamics of macromolecular assemblies allowing characterization of such biological processes (3,4). However, these methods, when applied to macromolecular assemblies, often suffer from low sensitivity. Dynamic Nuclear Polarization (DNP) is an emerging technique that provides dramatic sensitivity enhancements making it a promising tool to study low-concentration sites in the context of macromolecular assemblies. We present DNP-enhanced solid-state NMR studies of tubular assemblies of CA and CA-SP1. We demonstrate that sensitivity gains of 20-64 fold are attained in these assemblies. DNP-enhanced 2D and 3D spectra of CA and CA-SP1 exhibit many well-resolved regions permitting resonance assignments of an extensive number of residues, similar to our prior work with conical CA assemblies.[3] Resonance assignments were made for the majority of the signals of the SP1 region in CA-SP1, and the chemical shifts indicate that, at cryogenic temperatures, the SP1 region adopts a coil structure, similar to our prior findings at room temperature (4). The current results suggest that high-quality multidimensional correlation spectra can be obtained from HIV-1 tubular assemblies for their subsequent detailed structural characterization. This work was supported by the National Institute of Health (NIH Grant-P50GM082251). We acknowledge the support of the NSF CHE0959496 grant for acquisition of 850 MHz NMR spectrometer and of the NIGMS 1 P30 GM110758-01 grant for the support of core instrumentation infrastructure at the University of Delaware.

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F61. Coarse-Grained Simulation Reveals Key Features of HIV-1 Capsid Self-Assembly

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The spread of HIV-1 infection requires the release of a viral particle, or virion, from an infected cell. A newly-released virion is non-infectious, and so a sophisticated process of "maturation" occurs in order to produce a mature and infectious virion. One critical aspect of maturation is the self-assembly of many copies of the viral capsid protein to generate a cone-shaped capsid structure that surrounds the viral RNA: where a suitable capsid is not formed, the virion remains non infectious.

In order to study the critical early stages of capsid formation, we use a simplified "ultra-coarse-grained" (UCG) computational model of the capsid protein to examine capsid protein self-assembly behaviors in a range of environments relevant to the maturing HIV-1 virion. The simulation results suggest the critical importance of capsid protein concentration, molecular crowding, and dynamic changes in capsid protein structure for the nucleation and growth of a single mature viral capsid. These simulations elucidate important natural contributions to both on- and off-pathway capsid self-assembly processes, providing insights into aspects of virion maturation that are otherwise inaccessible to conventional experimental techniques.

F62. Structural Studies of HIV-1 Capsid CA Protein Assemblies: Insights into Structure by Magic Angle Spinning NMR Spectroscopy and Interactions with TRIM5α by Magic Angle Spinning NMR

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Human immunodeficiency virus 1 (HIV-1) infects vital cells in the human immune system. The capsid protein (CA) plays an essential role in the HIV-1 life cycle, assembling into a cone-shape structure comprised by ~1200 copies of CA,[1,2] to enclose the viral RNA genome during viral maturation. TRIM5α protein is a cytoplasmic protein, which binds to the assembled capsid to block HIV replication, by inducing premature capsid disassembly and recognition functionality.[3] To examine, at atomic resolution, the interactions of TRIM5α with the HIV-1 capsid, we employed magic angle spinning NMR spectroscopy. We have prepared tubular assemblies of CA protein using hexameric building blocks formed by cross linking of U-^{13}C,^{15}N-CA A14C/E45C/W184A/M185A mutant protein.[4] We have characterized these assemblies by a combination of 2D MAS NMR experiments. We have conducted structural studies of these CA tubular assemblies in complex with Rhesus monkey TRIM5α_{rh} CC-SPRY protein. We have detected multiple chemical shift and peak intensity perturbations upon the formation of the complex, indicative of conformational changes and possible allosteric effects. We have developed a new approach dubbed dREDOR for direct detection of binding interfaces formed by interacting proteins, where one binding partner in the complex is uniformly isotopically labeled with ^{13}C,^{15}N and another one is at natural abundance. We discuss the applications of this approach to analysis of binding interfaces in the complex formed by tubular assemblies of U-^{13}C,^{15}N-CA A14C/E45C/W184A/M185A and TRIM5α_{rh} CC-SPRY.

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BACK

F63. Structural Basis and Distal Effects of Gag Substrate Co-Evolution in Drug Resistance to HIV-1 Protease

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Drug resistance mutations in response to HIV-1 protease inhibitors are selected not only in the drug target but elsewhere in the viral genome, especially at the protease cleavage sites in the Gag polyprotein. To understand the molecular basis of this protease–substrate co-evolution, we solved the crystal structures of drug resistant I50V/A71V HIV-1 protease with p1-p6 substrates bearing coevolved mutations. Analyses of the protease–substrate interactions reveal that compensatory co-evolved mutations in the substrate do not restore interactions lost due to protease mutations, but instead establish novel interactions that are not restricted to the site of mutation. Mutation of a substrate residue has distal effects on other residues' interactions as well, including through the induction of a conformational change in the protease. Additionally, molecular dynamics simulations suggest that restoration of active site dynamics is an additional constraint in the selection of coevolved mutations. Hence, protease–substrate co-evolution permits mutational, structural, and dynamic changes via molecular mechanisms that involve distal effects contributing to drug resistance.

F64. Identifying Patterns of Correlated Resistance Mutations in HIV-1

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The focus of considerable bioinformatics research regarding the development of drug-resistance mutations in HIV has been to explain therapy outcomes via patterns of single resistance mutations or major-compensatory mutation pairs. While substantial insight has been gained through these studies, systematically identifying the effects of larger patterns of mutations on resistance from sequence data has been elusive. We have constructed statistical models called Potts models using the coevolutionary information encoded in publically available multiple sequence alignments of treated patients to explain the effects of networks of interacting resistance mutations (1,2). Potts models have been used to predict residue-residue contacts from sequence information (3,4), and the fitness landscapes of drug-naïve HIV proteins under immune pressure (5,6). These models, which we have currently parameterized using HIV-1 subtype B drug-experienced protease sequences, can 1.) accurately predict probabilities of observing mutation patterns undersampled in multiple sequence alignments; 2.) identify sequence patterns which differ in preference for acquiring resistance mutations; and 3.) recover intermediate mutational pathways that lead to resistance. Ultimately, we aim to build a robust statistical framework with which we can better understand the epistatic effects of the background sequence on the development of drug-resistance mutations in HIV proteins. As a preliminary study, we are focusing on patterns of major resistance sites A82V, I84, L90 and compensatory sites I10, L63, A71, and I93 (7).

These models require coevolution information from sequences, which is a priori lacking from deep sequencing data due to missing linkage between reads. However, we have previously developed a methodology to estimate pair correlations from deep sequencing data (8), and moving forward, this may be sufficient to construct Potts models of the sequence landscape within individual patients and associate specific patient therapy outcomes with mutation patterns observed using deep sequencing.

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BACK

F65. Co-Evolution of HIV-1 Protease and p1-p6 Modulates Gag Processing

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The ordered, sequential, proteolytic processing of viral Gag and GagPol polyproteins by HIV-1 protease (PR) plays an essential role in viral maturation. As a result, HIV-1 PR is an important target for anti-viral therapy, with several protease inhibitors (PI) in use clinically. However, the virus accumulates mutations within PR that not only render PIs less effective but also impair PR function. It is well established that correlated, compensatory mutations are selected within Gag and likely modulate processing by PR at the cleavage sites. Previously, we have shown that p1-p6 cleavage site mutations are co-evolved with the D30N/N88D NFV-resistance PR mutations. Structurally, mutations in the PR and/or the substrate alter interactions between PR residue 30 and p1-p6 substrate, which are compensated by the co-evolving L449F and S451N mutations in the p1-p6 cleavage site. In this study we examine if rate of cleavage at the different Gag cleavage sites by HIV-1 PR is altered as a result of mutations in PR and/or the p1-p6 cleavage site. Gag was synthesized using an *in vitro* coupled transcription-translation system in rabbit reticulocyte lysate and then processed by added PR, giving rise to intermediate species and finally, the end products. Processing of Gag variants with WT and D30N/N88D PR will reveal how PR–substrate co-evolution may alter the rate of cleavage at different sites and contribute to resistance.

BACK

F66. Viral Quasispecies and Mutational Analysis using Next-Generation Sequencing

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The development of resistance against HIV protease inhibitors has contributed to the persistence of the HIV/AIDS epidemic. While the role of drug resistance mutations in protease have been studied comprehensively, mutations in its substrate, Gag, have not been extensively cataloged. Next-Generation Sequencing (NGS) has transformed our understanding of the mutational landscape of human pathogens such as HIV and Hepatitis C Virus and model systems such as Flock House Virus and Sindbis Virus. I will describe computational analyses of NGS data, designed to examine co-evolution of HIV-1 Gag and protease in a large patient cohort. The serum or plasma patient samples were obtained when therapy failed to adequately suppress viral replication (generally 1,000 copies/mL), allowing multiple samples to be taken for some patients. By analysing mutational events such as nucleotide mismatches and RNA recombination, we find that the majority of these patients harboured virus populations with short duplications proximal to the proteolysis sites in the GAG protein after resistance to protease inhibitors had developed. We developed algorithms that populate large matrices corresponding to every possible pairwise interaction of nucleotides and/or recombination events in the HIV genome with the NGS data and use classical linkage disequilibrium statistics to reveal the correlated evolution of protease amino-acid substitutions with GAG proteolysis site duplications.

F67. Impact of the HLA B*57 Allele on Intra-Host HIV-1 Capsid Phylodynamics and RNA Secondary Structure Diversity

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The HLA-B*5701 allele, which targets a particular region within p24, has been associated with control of viral replication (Norstrom *et al.,* 2012). For this reason, viral protein evolution in patients with this specific allele has been studied in order to provide insights into modes of protection that are essential for the successful development of vaccine and/or treatment strategies. Until recently, little research has been undertaken to explore the selective pressures at the level of both the protein and the underlying RNA secondary structure, the combination of which likely results in a complex interplay between protein and RNA structural evolution required for productive infection. In particular, since HIV-1-infected subjects usually harbor at any given time a heterogeneous population of viral strains, or quasispecies, the belief of a single RNA structure has recently been challenged (Rife *et al.,* under review). Evidence in this study suggested the existence of a potentially functional intra-host population of RNA secondary structures, referred to by analogy as a "quasistructure". Based on these findings, we hypothesized that selective pressures at the protein level may affect the preservation of HIV-1 RNA quasistructures within an infected host. Using the existing SHAPE structure (Watts *et al.,* 2009) and a variety of phylodynamic and RNA structural prediction methods, we have attempted for the first time to understand how HIV-1 diversity at the nucleotide level is associated with RNA structural diversity over the course of infection and the importance of the HLA-B*57 allele on RNA quasistructure evolution. Viral population dynamics were inferred utilizing extensive longitudinal sequence analysis within the Bayesian statistical framework for six HIV-1-infected patients with and six without the B*57 allele. Both RNA-Decoder and Mfold programs were then used to determine intra-host RNA structural diversity over time. Although no clear correlation was found between changes in viral effective population size over time, a reflection of viral diversity, and temporal patterns in RNA secondary structure, patterns distinguishing patient cohorts were clearly evident. The most intriguing pattern was a statistically significant increase in the number of potential structures as well as increased thermodynamic stability of the inferred structures in subjects carrying the HLA-B*5701 allele. This allele-specific pattern may play an important role in the reduced viral replication observed within these patients, e.g. via ribosomal pausing, and potentially proving useful as a therapeutic target.

BACK

F68. Deep Mutational Scanning of the Overlapped HIV-1 Genes *Tat* and *Rev* Reveals a Selfish Segregation of Functional Motifs

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Some genes contain coding overlap – regions in which nucleotides are shared in alternative open reading frames and encode different proteins. These regions, present in most viruses, pose an interesting evolutionary dilemma as the same region of DNA must evolve under the joint selection pressures and functional requirements of both proteins. The viral regulatory genes *tat* and *rev* of Human Immunodeficiency Virus type 1 (HIV-1) provide an ideal system to explore this problem as both are essential in viral replication. Here we combine statistical analyses of patient data, a complete, residue-resolution, functional dissection of Tat and Rev, and directed evolution experiments that decouple the overlapped regions and generate all 12,928 viral point mutations to demonstrate that the surprising selfish organization of the *tat/rev* overlap minimizes the constraining effects of overlapped reading frames. Additionally, we find evidence that this genetic arrangement may provide evolutionary advantages by preferentially excluding deleterious mutations.

BACK

F69. Combining Chemical and Intra-Host Evolutionary Information for HIV-1 RNA Secondary Structure Prediction

Brittany D. Rife[1,2], Gary B. Fogel[3], Enoch Liu,[3] Susanna L. Lamers[4], David J. Nolan[1,2], Melissa Norstrom[5], Frederick M. Hecht[6], Annika C. Karlsson[5], and Marco Salemi[1,2]

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Non-coding RNA (ncRNA) structures within HIV-1 are known to play important roles in regulation, yet a large proportion of predicted RNA structures within HIV-1 protein-coding regions remain uncharacterized. Differences in evolutionary parameters, such as transition/transversion ratios, substitution rates, and nucleotide composition, in paired versus unpaired regions have been described as evolutionary evidence of structure within ncRNAs; however, in the case of protein-coding RNA (cRNA) of the HIV-1 capsid (*p24*), disagreement between these evolutionary patterns and previously reported SHAPE-based RNA structure suggests that there is additional complexity when attempting to resolve RNA structures in protein-coding regions. For this study, *in vivo* evolutionary patterns of *p24* sequences collected longitudinally, from early infection up to seven years, from treatment-naïve subjects were mapped onto the previously predicted *p24* RNA secondary structure. We show that while chemical probing and biophysical methods can provide valuable insight into RNA folding and thermostability, they are insufficient for resolving accurate RNA structures that reflect the evolutionary dynamics of rapidly evolving viruses with genetically distinct intra-host variants experiencing selective pressure at the levels of both RNA and protein. However, taking into account both chemical (SHAPE) and phylogenetic (pairing probability) data can provide insight into potential discrepancies in structure prediction methods based on chemical data from a single sequence. In addition, the use of this combined approach provides a clearer picture of the more highly conserved structured sub-regions among heterogeneous viral sequences, the preservation of which may be masked by the diversity of surrounding substructures when analyzed globallyusing common methods of RNA structure prediction. This combined approach is currently being developed as a program that utilizes RNA structure single-site restraints based on user-specified threshold values of SHAPE reactivity and RNA-Decoder-derived pairing probabilities and is specifically designed for cRNA sequences.

BACK

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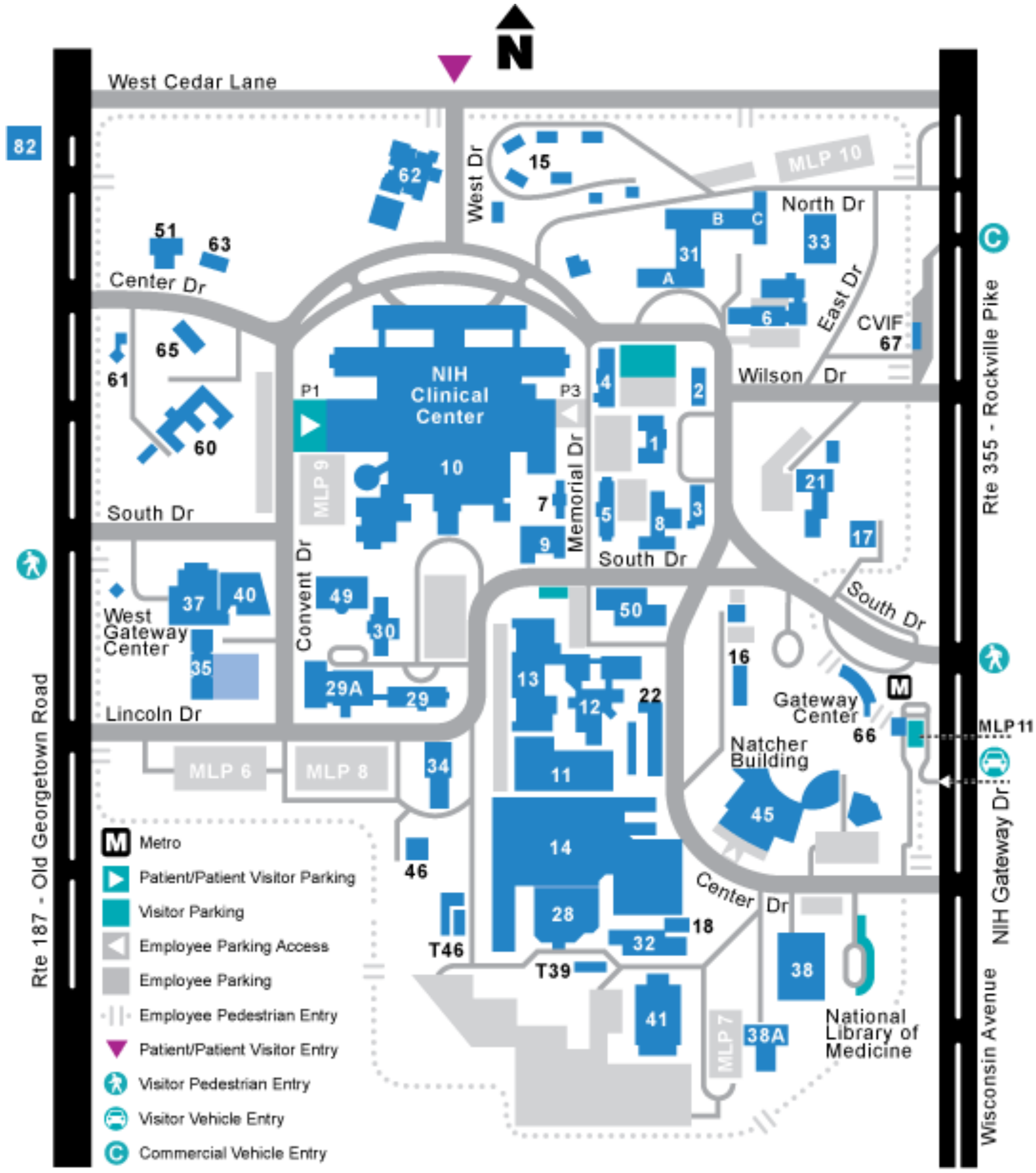
BACK

Map of NIH Campus



Some useful notes:

- The conference is located in Natcher (Building 45), southwest of the Gateway Center entrance to campus. The Medical Center Metro stop is next to the Gateway Center.
- Non-NIH employees must undergo inspection and receive temporary ID cards at the Gateway Center (see additional information on following pages).
- Parking garage MLP-11 is for non-NIH employees; NIH employees may park in the garage under Building 45 (requires car safety inspection next to Building 38A) or in other employee parking.
- Dining options on the NIH campus include Eurest Dining Services locations in Buildings 45, 1, 10, and 31, Maryland Business Enterprise Program for the Blind locations in Buildings 38A and 12B, and a concession stand in the Natcher lobby (http://does.ors.od.nih.gov/food/index.htm).
- An ATM is located in the Natcher lobby.

BACK

NIH Gateway Center Map

Main Visitor Entrance: NIH Gateway Drive
Gateway Center - Building 66 (for pedestrians entering campus)

Gateway Inspection Station - Building 66A (for vehicles entering campus)

- Monday – Friday: 5am – 10pm; Weekends and After Hours: Closed After hours: After 10pm on weekdays, all day weekends and holidays, pedestrians and visitors in vehicles should enter campus via the Commercial Vehicle Inspection Facility (CVIF) - Building 67 (on Rockville Pike between North Drive and Wilson Drive)
- After inspection, vehicles enter campus at Center Drive
- Roadway at Center Drive is for entering campus only; visitors exiting campus may exit from other open locations. To see a list of exits, please see the map.
- All vehicles and their contents will be inspected upon entering the campus.

Multi-Level Parking Garage 11 – MLP-11 (for parking outside of campus)

• Monday – Friday: 6am – 9pm (entrance) 6am – 11pm (exit) Weekends: Closed
• When MLP-11 is closed, visitors can park in lots on the NIH Campus
• Visitors parking in this garage should proceed to the Gateway Center (Bldg. 66) to get a visitor badge
• All visitors traveling in a vehicle are highly encouraged to park in MLP-11 as there is limited visitor parking on the main campus
• No vehicle inspection required to park in MLP-11
• Vehicles left in the MLP-11 parking garage after 11pm on weekdays or during any weekends are subject to ticketing and towing
• Cost: $2 per hour for the first three hours, $12 maximum for the entire day

Directions to NIH Gateway Drive from Rockville Pike/Wisconsin Avenue:
Southbound:

1. Continue on Rockville Pike past South Drive
2. Turn right at NIH Gateway Drive

Northbound – Option 1:

1. Continue on Rockville Pike past South Drive
2. Make a u-turn from the left turn lane at Wilson Drive
3. Continue southbound on Rockville Pike past South Drive
4. Turn right at NIH Gateway Drive

Northbound – Option 2:

1. Continue on Rockville Pike
2. Turn left at Battery Lane
3. Turn right on Old Georgetown Road
4. Turn right on Cedar Lane
5. Turn right on Rockville Pike
6. Continue southbound on Rockville Pike past South Drive
7. Turn right at NIH Gateway Drive

Northbound – Option 3:

1. Continue on Rockville Pike to South Drive
2. Make a u-turn from the left turn lane at South Drive
3. Continue southbound on Rockville Pike
4. Turn right at NIH Gateway Drive

Security Procedures for Entering the NIH Campus:
* All visitors and patients—**please be aware**: Federal law prohibits the following items on Federal property: firearms, explosives, archery equipment, dangerous weapons, knives with blades over 2 ½ inches, alcoholic beverages and open containers of alcohol.
* The NIH has implemented security measures to help ensure the safety of our patients, employees, guests and facilities. All visitors must enter through the NIH Gateway Center at Metro or the West Gateway Center. You will be asked to submit to a vehicle or personal inspection.
* Visitors over 15 years of age must provide a form of government-issued ID such as a driver's license or passport. Visitors under 16 years of age must be accompanied by an adult.

Vehicle Inspections – All vehicles and their contents will be inspected upon entering the campus. Additionally, all vehicles entering certain parking areas will be inspected, regardless of any prior inspection. Drivers will be required to present their driver's license and may be asked to open the trunk and hood. If you are physically unable to perform this function, please inform the inspector and they will assist you.
Vehicle inspection may consist of any combination of the following: Detection Dogs Teams (K-9), Electronic Detection Devices and Manual Inspection.
After inspection, you will be issued a vehicle inspection pass. It must be displayed on your vehicle's dashboard while you are on campus. The inspection pass is not a "parking permit." It only grants your vehicle access to enter the campus. You can only park in designated parking areas.

Personal Inspections – All visitors should be prepared to submit to a personal inspection prior to entering the campus. These inspections may be conducted with a handheld monitoring device, a metal detector and by visible inspection. Additionally, your personal belongings may be inspected and passed through an x-ray machine.

Visitor passes must be prominently displayed at all times while on the NIH campus.
To learn more about visitor and security issues at the NIH, visit:
http://www.nih.gov/about/visitor/index.htm.
For questions about campus access, please contact the ORS Information Line at orsinfo@mail.nih.gov or 301-594-6677, TTY - 301-435-1908.